



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US98/24913 <b>(22) International Filing Date:</b> 24 November 1998 (24.11.98) <b>(30) Priority Data:</b> 08/977,493 24 November 1997 (24.11.97) US <b>(71) Applicant (for all designated States except US):</b> THE SCRIPPS RESEARCH INSTITUTE [US/US]; 10550 North Torrey Pines Road, La Jolla, CA 92037 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BOGER, Dale, L. [US/US]; 2819 Via Posada, La Jolla, CA 92037 (US). GILULA, Norton, B. [US/US]; 11 E. Roseland Drive, La Jolla, CA 92037 (US). LERNER, Richard, A. [US/US]; 7750 E. Roseland Drive, La Jolla, CA 92037 (US). CRAVATT, Benjamin, F. [US/US]; 3435 Lebon Drive #1024, San Diego, CA 92122 (US). <b>(74) Agents:</b> NORTHRUP, Thomas, E. et al.; The Scripps Research Institute, 10550 North Torrey Pines Road, TPC-8, La Jolla, CA 92037 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> INHIBITORS OF GAP JUNCTION COMMUNICATION  <b>(57) Abstract</b>  Oleamide is an endogenous fatty acid primary amide that possesses sleep-inducing properties in animals and has been shown to effect serotonergic systems and block gap junction communication in a structurally specific manner. Certain agents can serve both as an oleamide agonist and as an inhibitor of fatty acid amide hydrolase. Fatty acid amide hydrolase is responsible for the rapid inactivation of oleamide <i>in vivo</i> . The structural features of oleamide required for inhibition of gap junction-mediated chemical and electrical transmission in rat glial cells are defined. Effective inhibitors fall into two classes of fatty acid primary amides of which oleamide and arachidonamide are the prototypical members. Of these two, oleamide constitutes the most effective and its structural requirements for inhibition of the gap junction are well defined. It requires a chain length of 16–24 carbons of which 16–18 carbons appears optimal, a polarized terminal carbonyl group capable of accepting but not necessarily donating a hydrogen bond, a $\Delta^9$ cis double bond, and a hydrophobic methyl terminus. Within these constraints, a range of modifications are possible, many of which may with enhanced <i>in vivo</i> properties.		

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## INHIBITORS OF GAP JUNCTION COMMUNICATION

Technical Field:

The present invention relates to compounds that serve both  
5 as oleamide agonists and as inhibitors of fatty acid amide  
hydrolase. More particularly, the present invention relates to  
oleamide agonists for inhibition of gap junction-mediated  
chemical and electrical transmission in glial cells.

10 Background:

Oleamide (1) is an endogenous fatty acid primary amide  
shown to accumulate in the cerebrospinal fluid under conditions  
of sleep deprivation and disappear upon sleep recovery (Cravatt  
et al. (1995) *Science* 268, 1506-1509; Lerner et al. (1994) *Proc.*  
15 *Natl. Acad. Sci. USA* 91, 9505-9508; Cravatt et al. (1996) *J. Am.*  
*Chem. Soc.* 118, 580-590). In a structurally specific manner, it  
has been shown to induce physiological sleep in animals when  
administered by ip or iv injection. Consistent with its role as  
a prototypical member of a new class of biological signaling  
20 molecules, enzymatic regulation of the endogenous concentrations  
of oleamide has been described or proposed.

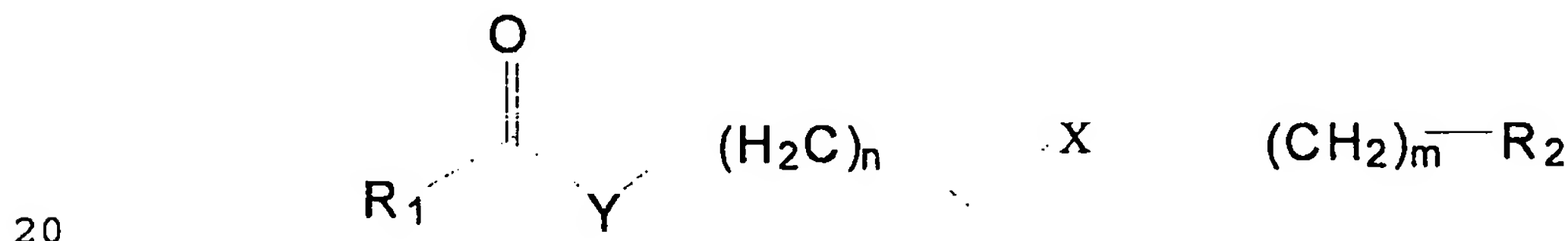
Fatty acid amide hydrolase (FAAH, oleamide hydrolase) is an  
integral membrane protein that degrades 1 to oleic acid and  
potent inhibitors ( $K_i = 13 \mu\text{M}^{-1} \text{ nM}$ ) of the enzyme with sleep-  
25 inducing properties have been detailed. The purification,  
characterization, cloning, expression and neuronal distribution  
of FAAH have been disclosed and it was found to possess the  
capabilities of hydrolyzing a range of fatty acid amides  
including anandamide which serves as an endogenous ligand for  
30 cannabinoid receptors.

In contrast to anandamide, an appealing feature of the  
members of this new class of biological signaling agents is the  
primary amide suggesting that their storage and release may be  
controlled in a manner analogous to that of short peptide

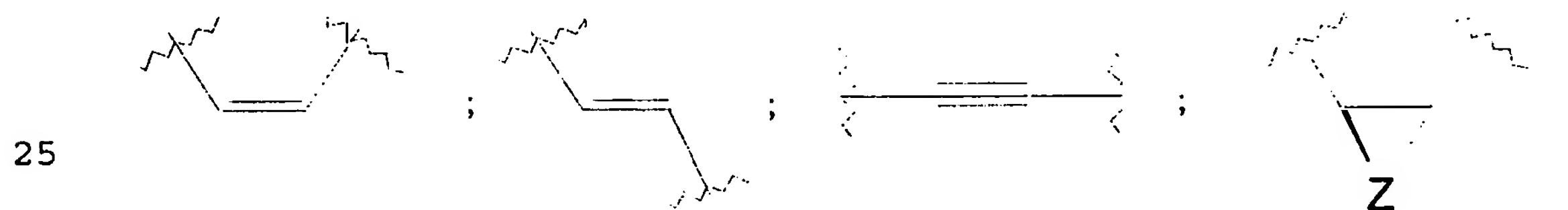
hormones and messengers terminating in a primary amide  
 (Patterson et al. (1996) *J. Am. Chem. Soc.* **118**, 5938-5945;  
 Cravatt et al. (1996) *Nature* **384**, 83-87; Giang et al. (1997)  
*Proc. Natl. Acad. Sci. USA* **94**, 2238-2242; Merkler et al. (1996)  
 5 *Arch. Biochem. Biophys.* **330**, 430-434; Devane et al. (1992)  
*Science* **258**, 1946-1949; Johnson et al. (1993) *Prostaglandins*,  
*Leukot. Essent. Fatty Acids* **48**, 429-437; Di Marzo et al. (1995)  
*Prostaglandins, Leukot. Essent. Fatty Acids* **53**, 1-11).

# 10 Summary of the Invention:

The invention is directed to inhibitors of gap junction  
 communication. More particularly, one aspect of the invention  
 is directed to compounds having oleamide agonist activity for  
 inhibiting gap junction-mediated chemical and electrical  
 15 transmission in glial cells. Preferred compounds are  
 represented by the structure:



In the above structure, X is one of the diradicals represented  
 by the following structures:



wherein Z is either -CH or O. Y is a diradical selected from  
 the the following: -CH<sub>2</sub>-, -CH(CH<sub>3</sub>)-, -C(CH<sub>3</sub>)<sub>2</sub>-, -O-, -NH-, -  
 CH(SH)-, -CHSAc)-, -CH(OH)-, -CHCl-, -C(=O)-, -C(=O)CH<sub>2</sub>-, -  
 30 CH<sub>2</sub>NHC(=O)-, or -CH<sub>2</sub>N(CH<sub>3</sub>)C(=O)-. R<sub>1</sub> is a radical selected from  
 the following: hydrogen, -NH<sub>2</sub>, OH, MeNH-, Me<sub>2</sub>N-, EtNH-, Et<sub>2</sub>N-,  
 CH<sub>2</sub>=CHCH<sub>2</sub>NH-, n-propyl-NH-, i-propyl-NH-, cyclopropyl-NH-, i-  
 propyl-NMe-, butyl-NH-, pyrrolidine-, phenyl-NH-, phenyl(CH<sub>2</sub>)<sub>3</sub>NH-  
 , HONH-, MeONMe-, NH<sub>2</sub>NH-, CH<sub>3</sub>O-, CH<sub>3</sub>CH<sub>2</sub>O-, CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>O-, Me<sub>2</sub>CHCH<sub>2</sub>O-,



H-, CF<sub>3</sub>-, BrCH<sub>2</sub>-, ClCH<sub>2</sub>-, N<sub>2</sub>CH-, HOCH<sub>2</sub>CH<sub>2</sub>NH-, (HOCH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N-, HOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH-, or HOCH<sub>2</sub>CH(OAc)CH<sub>2</sub>O-. R<sub>2</sub> is a radical selected from the following:

-CH<sub>3</sub>, -(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>, -(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>, -(CH<sub>2</sub>)<sub>6</sub>CH<sub>3</sub>, -CH<sub>2</sub>OCH<sub>3</sub>, -CH<sub>2</sub>OH, -CONH<sub>2</sub> or  
 5 -CO<sub>2</sub>H. n is an integer from 0 to 15; m is an integer from 0 to 15 with the requirement that the sum of n+m is an integer from 11 to 15. However, if Y is CH<sub>2</sub>, n is 4, m is 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub> cannot be a radical selected from the group consisting of -CF<sub>3</sub> and hydrogen. Furthermore, if Y is CH<sub>2</sub>, n is 5, m is 7, and  
 10 R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub> cannot be a radical selected from the group consisting of -CF<sub>3</sub>, -CH<sub>2</sub>Cl, -NHOH, -C(O)NH<sub>2</sub>, -CN<sub>2</sub>, and -C(O)OEt. If Y is CHCl, n is 4, m is 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub> cannot be NH<sub>2</sub>. If Y is CH(OH), n is 4, m is 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub> cannot be NH<sub>2</sub>. If Y is C(=O), n is 4, m is 7, and R<sub>2</sub> is CH<sub>3</sub>,  
 15 then R<sub>1</sub> cannot be NH<sub>2</sub> and CH<sub>3</sub>CH<sub>2</sub>O-. And finally, if Y is CH<sub>2</sub>, 4 ≤ n ≤ 9, 4 ≤ m ≤ 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub> cannot be NH<sub>2</sub> and OH.

Another aspect of the invention is directed to a method for inhibiting gap junction mediated chemical and electrical transmission in glial cells by contacting such cells with the  
 20 oleamide agonists represented above.

#### Description of Figures:

Figure 1 shows a structure of the sleep inducing lipid, oleamide (*cis*-9-octadecenamide, 18:1<sup>9</sup>).

25 Figure 2 illustrates fatty acid primary amide analogs with % inhibition of gap junction-mediated dye transfer wherein the indicated footnotes are represented as follows: (a) Chain length:number of double bondsposition of double bond, the double bond stereochemistry is *cis* unless indicated otherwise. (b) %  
 30 Inhibition of lucifer yellow dye transfer by rat glial cell gap junction at the indicated concentration of agent. (c) Low solubility precluded testing at higher concentration. (d) Not done (For 22:1<sup>9</sup>, see Figure 18 for synthesis).

Figure 3 illustrates carboxamide terminus analogs with %

inhibition of gap junction-mediated dye transfer wherein the indicated footnotes are represented as follows: (a) inhibition of GAP junction-mediated dye transfer, see Figure 2; (b) Not done; (c) Toxic to cells at concentration tested; (d) Tested at 25 mM.  
5 (e) Insoluble at concentration tested. (f) Reversible inhibition.

Figure 4 illustrates ethanolamide substituted analogs with % inhibition of gap junction-mediated dye transfer wherein the indicated footnotes are represented as follows: (a) Inhibition  
10 of GAP junction-mediated dye transfer, see Figure 2; (b) Not done; (c) Racemic and (2*S*)-1-oleyl-2-acetylglycerol.

Figure 5 illustrates methyl terminus substituted analogs with % inhibition of gap junction-mediated dye transfer wherein the indicated footnotes are represented as follows: (a)  
15 Inhibition of GAP junction-mediated dye transfer, see Figure 2 and Figure 19 for further data and synthetic routes for some of these compounds.

Figure 6 illustrates *N*-Oleyl Glycine substituted analogs with % inhibition of gap junction-mediated dye transfer wherein  
20 the indicated footnotes are represented as follows: (a) Inhibition of GAP junction-mediated dye transfer, see Figure 2; (b) Not done; (c) Low solubility.

Figure 7 illustrates olefin modification analogs with % inhibition of gap junction-mediated dye transfer wherein the  
25 indicated footnotes are represented as follows: (a) inhibition of GAP junction-mediated dye transfer, see Figure 2 (b) Not done; (c) Low solubility precluded testing at higher doses; see Figure 20 for synthetic routes for some of these compounds.

Figure 8 illustrates linker modification substituted  
30 analogs with % inhibition of gap junction-mediated dye transfer wherein the indicated footnotes are represented as follows: (a) Inhibition of GAP junction-mediated dye transfer, see Figure 2; (b) Not done; (c) Tested at 150 mM; (d) Reversible; see Figures 21 and 22 for synthetic routes for some of these compounds.

Figure 9 illustrates the effects of different agents on calcium wave propagation and gap junctional communication. The number of cells participating in calcium wave propagation was quantified by counting the number of responding cells in one  
5 direction from the stimulated cell. The time points indicated above were analyzed after the initiation of calcium signaling; the responding cell numbers are expressed as Mean  $\pm$  SD. Gap Junction dye transfer was expressed as dye coupling percentage (Mean  $\pm$  SD) which is described in the assay procedures. Each  
10 treatment was performed in 3 dishes, and 10 individual fields were examined in each dish, N = 30.

Figure 10 illustrates oleamide inhibition of gap junction mediated dye coupling between rat glial cells. Cultured rat glial cells (A and D, phase) were microinjected with Lucifer  
15 yellow (B and E) and showed efficient dye transfer to adjacent cells under (C) control conditions (0.1% ethanol present in culture media). (F) Treatment of glial cells with 50  $\mu$ M oleamide for 10 minutes completely blocked dye transfer. Glial  
cells were also scrape loaded (G, phase) with Lucifer yellow,  
20 and under control conditions dye was efficiently transferred from dye loaded cells to adjacent cells (H), with no dye spread to adjacent cells when treated with oleamide (I). Scale bar = 50  $\mu$ m.

Figure 11 illustrates: (J) Dose-response studies of  
25 oleamide-induced blockage of dye transfer were conducted by treating glial cells with various concentrations of oleamide (5-100  $\mu$ M) for 4 h prior to injection of Lucifer yellow and monitoring of dye transfer. Approximately 50% inhibition of dye transfer was observed with 20  $\mu$ M oleamide, and complete  
30 inhibition of dye transfer was observed with 50  $\mu$ M oleamide. (K) Time course of oleamide-induced blockage of gap junction dye transfer was determined by treating glial cells with 50  $\mu$ M oleamide for indicated times prior to injection of Lucifer yellow. Reversibility of oleamide's effect on gap junctions was

established by removing oleamide-containing media from the culture dish, reculturing cells with fresh media without oleamide, and monitoring dye transfer over the subsequent hour. Complete recovery of dye transfer to control levels was observed  
5 within one hour after removal of oleamide.

Figure 12 illustrates double whole cell patch recording combined with observation of dye spread was used to assess gap junctional conductance in cultured rat glial cells. (A) Time course of voltages applied to each cell. Cells were held at -40  
10 mV and first cell 1, then cell 2 was stepped to -20 mV. Thick trace shows voltage applied to cell 1, thin trace the voltage applied to cell 2. (B) and (C), Response of cells to the voltage protocol shown in (A). Thick traces show the currents in cell 1, the thin traces show currents in cell 2. The upward  
15 current deflections show the sum of surface membrane and junctional current in the cell whose potential is changed from -40 mV to -20 mV. The upward deflections from 50 msec to 150 msec are currents in cell 1, and the deflections from 200 to 300 msec are currents in cell 2. Downward deflections consist  
20 solely of junctional currents recorded in the cell held at -40 mV. In (B), large downward current deflections show a pair of control cells that was well coupled. Note that although the upward deflections elicited by pulsing first one cell, then the other, are different sizes, the downward current deflections,  
25 elicited by a 20 mV transjunctional voltage, are of equal size, demonstrating that a driving force of 20 mV produces a constant junctional current regardless of which cell is pulsed. In (C), the lack of downward current deflections in response to a 20 mV pulse shows that a different pair of cells exposed to 50  $\mu$ M  
30 oleamide was completely uncoupled. Panel (D) compares junctional currents of control and experimental cell pairs during the first 150 msec of the measurements shown in (B) and (C). Note that there is no detectable downward deflection in the trace from the cell pair exposed to oleamide. In this case,

the data set an upper limit on the residual junctional conductance of less than 50 pS. Thus, the junction is completely uncoupled. In measurements carried out on eight cell pairs the mean junctional conductance of control pairs was  $13 \pm 7$  nS (n=3) and the mean junctional conductance of cell pairs exposed to 50  $\mu$ M oleamide was  $0.5 \pm 0.7$  nS (n=5).

Figure 13 illustrates structure-activity studies of oleamide-induced blockage of gap junction dye transfer were conducted to evaluate which chemical features of oleamide were required for its inhibitory effect. Oleic acid and *trans*-9-octadecenamide had no effect on dye transfer, while of the remaining *cis*-monounsaturated fatty acid amides, oleamide proved to be the most potent inhibitor. Following oleamide in order of potency were: *cis*-8-octadecenamide > oleyl ethanolamide > *cis*-11-octadecenamide. Oleamide was also compared to other gap junction inhibitors, anandamide, 18 $\beta$ -GA and heptanol.

Figure 14 illustrates oleamide effect on gap junction dye transfer between BHK/ $\beta$ 1 cells. In the presence of zinc induction, BHK/ $\beta$ 1 cells (A and C, phase) were microinjected with Lucifer yellow and showed efficient dye transfer to adjacent cells in the control condition (B) (with 0.1% ethanol in culture media) and no dye transfer between cells that were treated with oleamide (D) (50  $\mu$ M oleamide for 10 min).

Figure 15 illustrates: (E) Comparison of dye transfer rates between BHK/ $\beta$ 1 cells under different treatment conditions. BHK/ $\beta$ 1 cells without zinc induction had a low incidence (10.2 $\pm$ 4.2%) of dye transfer. After adding zinc to the culture medium for 8-18 hr, dye transfer was significantly increased (95 $\pm$ 4.5%). 50  $\mu$ M oleamide completely blocked dye transfer (0.31 $\pm$ 0.24%) in the zinc induced sample. Anandamide (50  $\mu$ M), 18 $\beta$ -GA (50  $\mu$ M), and heptanol (3 mM) showed similar inhibition, while oleic acid (50  $\mu$ M) and *trans*-9-octadecenamide (50  $\mu$ M) had no effect on dye transfer (E). All determinations were made following 4 hours of treatment. Scale bar = 50  $\mu$ M.



Figure 16 illustrates effect of oleamide and 18 $\beta$ -GA on calcium wave propagation in rat glial cells. Rat glia (phase, A) were loaded with the calcium-indicator dye, Fluo-3, and changes in the intracellular free calcium concentration ([Ca<sup>2+</sup>]<sub>i</sub>) were documented as changes in the intensity of fluorescent dye both prior to (B) and after (C-F) mechanical stimulation. Fluorescence changes were monitored with an inverted fluorescence microscope. In the presence of 40  $\mu$ M 18 $\beta$ -GA (incubated with glia for 10 minutes prior to mechanical stimulation), mechanical stimulation resulted in an increase in [Ca<sup>2+</sup>]<sub>i</sub> in the stimulated cell, but this [Ca<sup>2+</sup>]<sub>i</sub> change did not propagate to any other cells (C). In contrast, in rat glia treated with 50  $\mu$ M oleamide (preincubation from 10 minutes to 4 hours) mechanical stimulation produced an increase in [Ca<sup>2+</sup>]<sub>i</sub> that was rapidly propagated to cells at long distances in an indistinguishable manner from control cell populations (D-F, time course of wave propagation; D, 2 sec; E, 4 sec; F, 8 sec). Scale bar = 30  $\mu$ m. For a quantitative comparison of calcium wave propagation in cells treated by different agents and control cell populations, see Figure 9.

Figure 17 illustrates western blot analysis of the  $\alpha$ 1 connexin protein from glia treated with oleamide and its inactive structural analogs. Lane 1 contains a sample of control cells treated with 0.1% ethanol for 4 hours. Lanes 2-4 contain samples of cells treated for 4 hours with 50  $\mu$ M oleic acid, 50  $\mu$ M trans-9-octadecenamide, and 50  $\mu$ M oleamide, respectively. The loss of P2  $\alpha$ 1 in glia treated with oleamide was reversible, as P2 was present at control levels after removal of oleamide from the culture dish and culturing the cells in normal culture media for 4 hours (lane 5). The nonphosphorylated (NP) and phosphorylated (P1 and P2)  $\alpha$ 1 connexin isoforms and molecular weight standards are indicated on the right of the blot.

Figure 18 illustrates a general synthesis for oleamide and analogs.

Figure 19 illustrates various syntheses for analogs including a Wittig type approach for the synthesis of analogs  
5 (see scheme 5 for data for these compounds).

Figure 20 illustrates a general synthesis of olefin derivatives (see scheme 7 for data for these compounds).

Figure 21 illustrates the synthesis of various  $\alpha$ -methyl derivatives (see scheme 8 for data for these compounds).

10 Figure 22 illustrates the synthesis of various  $\alpha$ -sulfhydryl derivatives (see scheme 8 for data for these compounds).

#### Detailed Description:

The invention relates to compounds that serve both as  
15 oleamide agonists and as inhibitors of fatty acid amide hydrolase. More particularly, the present invention relates to oleamide agonists for inhibition of gap junction-mediated chemical and electrical transmission in glial cells.

Our finding that oleamide inhibits the gap junction-  
20 mediated intercellular chemical and electrical transmission without effecting calcium wave propagation in rat glial cells suggests this may constitute an important site of action for this new class of biologically active lipids. The effective inhibitors of the gap junction fall into two classes of fatty  
25 acid primary amides of which oleamide and arachidonamide are the prototypical members. Of these, oleamide constitutes the most effective and its structural requirements for inhibition of the gap junction are well defined. The inhibition requires a chain length of 16-24 carbons of which 16-18 appears optimal, a  
30 polarized terminal carbonyl capable of accepting but not necessarily donating a hydrogen bond, a  $\Delta^9$  cis double bond, and a hydrophobic methyl terminus. Within these constraints, a range of modifications are acceptable, many of which may be utilized to enhance in vivo properties. In addition to those



which would slow or avoid inactivation by FAAH hydrolysis, a set of especially interesting agents have been identified which serve both as oleamide agonists and as FAAH inhibitors.

The inhibition of the gap junction by oleamide is  
5 consistent with a number of mechanisms. Perturbations in the bulk membrane fluidity or the membrane-protein interface that would effect the conformation of the membrane bound proteins as well as direct interaction with the gap junction proteins have been discussed (Lars Bastiaanse et al. (1993) *J. Membrane Biol.*  
10 136, 135-145). Similarly, we are looking at both progressive closure and ultimately collapse of the gap junction as well as gated closure of the gap junction. The compounds disclosed herein and the defined structural features of oleamide responsible for the properties may help distinguish among the  
15 mechanisms as well as provide new neuromodulatory agents applicable to sleep or mood disorders, analgesia, and disorders associated with higher neuronal function.

Example 1: Chemical Requirements and design of Analogs for  
20 Inhibition of Gap Junction Communication by the Biologically Active Lipid Oleamide

In addition to its sleep-inducing properties in animals, oleamide has been shown to effect serotonergic systems (Huidobro-Toro et al. (1996) *Proc. Natl. Acad. Sci. USA* 93,  
25 8078-8082) and block gap junction communication in a structurally specific manner. Moreover, while it was found to inhibit the gap junction-mediated chemical and electrical transmission in rat glial cells, it had no inhibitory effect on mechanically-stimulated or glutamate-induced calcium wave  
30 propagation thereby decoupling two previously indistinguishable glial cell communication pathways. Given the central role of intercellular chemical and electrical signaling in the CNS, the oleamide inactivation of glial cell gap junction channels may influence higher order neuronal function including sleep.

**Fatty Acid Primary Amide analogs.** The first series of compounds examined were the primary amides of the naturally occurring fatty acids and related synthetic analogs, Figure 2 (Arafat et al. (1989) *Life Sci.* **45**, 1679-1687; Wakamatsu et al. (1990) *Biochem. Biophys. Res. Commun.* **168**, 423-429; Jain et al. (1992) *J. Med. Chem.* **35**, 3584-3586). We have examined the number, position, and stereochemistry of the double bond(s), and the length of the agent. From these studies, which were assisted with the inclusion of the primary amides of nonnaturally occurring fatty acids, a clear depiction of the structural requirements of the endogenous agent emerged. The effective inhibitors of the gap junction-mediated dye transfer fall into two classes of which oleamide and arachidonamide are the prototypical members (Pinto et al. (1994) *Mol. Pharmacol.* **46**, 516-522; Felder et al. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 7656-7660; Abadji et al. (1994) *J. Med. Chem.* **37**, 1889-1893; Adams et al. (1995) *J. Pharmacol. Exp. Therapeut.* **273**, 1172-1181; Adams et al. (1995) *Life Sci.* **56**, 2041-2048; Priller et al. (1995) *Mol. Pharmacol.* **48**, 288-292; Khanolkar et al. (1996) *J. Med. Chem.* **39**, 4515-4519; Venance et al. (1995) *Nature* **376**, 590-594).

For agents related to oleamide that contain one double bond, the presence, position, and stereochemistry of the olefin as well as the chain length were found to have a defined relationship. The monounsaturated fatty acid primary amides of 16-24 carbons containing a  $\Delta^9$  cis double bond were all found to be effective inhibitors of the gap junction-mediated dye transfer. Removal of the  $\Delta^9$  double bond (18:0) or its replacement with a trans double bond (18:1<sup>9-trans</sup>) resulted in no observable inhibition at the concentrations examined. Shortening the chain length to 14 carbons (14:1<sup>9</sup>) resulted in the loss of activity while lengthening the chain slightly diminished the inhibitory effectiveness. No further substantial trend in

the length was observed with the longer fatty acid primary amides provided they possess a  $\Delta^9$  cis double bond:  $14:1^9 \ll 16:1^9 = 18:1^9 > 20:1^9 = 22:1^9 = 24:1^9$  (i.e. chain length:position of double bond) (Figure 2).

5       The position of the cis double bond had a well-defined effect on the potency. A  $\Delta^9$  cis olefin exhibited the strongest inhibitory effect and the potency typically declined as its position was moved in either direction. This is apparent in the comparisons made in oleamide series (18:1) and especially clear  
10 in the eicosenoamide series (20:1) where the potency declined as the distance from the  $\Delta^9$  position increased. Although this location in oleamide is central to its structure and potentially represents a relationship with either the carboxamide or methyl terminus, the potent activity of palmitoleamide (16:1<sup>9</sup>) suggested  
15 that it is the positional relationship with the amide that is important.

This was confirmed in the 20:1, 22:1, and 24:1 series where inhibitory activity was observed preferentially with the synthetic  $\Delta^9$  olefins rather than the naturally occurring isomers  
20 bearing a cis double bond 9 carbons from the methyl terminus:  $20:1^9 = 20:1^{11}$ ,  $22:1^9 > 22:1^{13}$  and  $24:1^9 > 24:1^{15}$ . In this context, it is interesting that erucamide (Hasler et al. (1991) *Am. J. Physiol.* 261 (Cell Physiol. 30), C161-C168), which has been detected in human cerebrospinal fluid, is not an inhibitor of  
25 the gap junction and does not induce physiological sleep in rats (Figure 2).

With the polyunsaturated fatty acid primary amides, those containing two double bonds exhibited modest activity. Typically, this was considerably diminished relative to that of  
30 oleamide except linoleamide (18:2) which also possesses the cis  $\Delta^9$  double bond. Even with linoleamide, the inhibition potency was lower than oleamide indicating that the additional  $\Delta^{12}$  cis double bond was not beneficial (Figure 2).

The primary amide of fatty acids containing three double

bonds were even less active with essentially all members being ineffective at 50  $\mu$ M. This includes  $\alpha$ -linolenamide (20:3<sup>9,12,15</sup>) which incorporates the  $\Delta$ 9 cis double bond of oleamide and 20:3<sup>8,11,14</sup> which incorporates an alternative  $\Delta$ 8 or  $\Delta$ 11 cis double  
5 bond. The exception to this generalization is  $\gamma$ -linolenamide (18:3<sup>6,9,12</sup>) which proved to be an effective but less potent inhibitor. Similarly, arachidonamide containing four cis double bonds was an effective inhibitor of the gap junction-mediated dye transfer although it was not quite as potent as  
10 oleamide. This effect was also observed with a fatty acid primary amide containing five cis double bonds but was lost again with that possessing six double bonds (Figure 2).

These results are consistent with two classes of fatty acid primary amides that inhibit the gap junction-mediated dye  
15 transfer. The prototypical member of the first, more potent class is oleamide and the second, slightly less effective class, contains arachidonamide (Figure 2).

**Carboxamide analogs.** An extensive study of the requirements at the carboxylate terminus proved revealing in  
20 that it was surprisingly tolerant, Figure 3. Oleic acid was found to be inactive at the concentrations examined, and oleyl alcohol, methyl or ethyl oleate, and oleyl acetate exhibited progressively reduced effectiveness. Oleic acid (Hirschi et al. (1993) *Am. J. Physiol.* 265 (*Cell Physiol.* 34), C1517-C1526; Burt  
25 et al. (1991) *Am. J. Physiol.* 260 (*Cell Physiol.* 29), C439-C448), arachidonic acid (Giaume et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 5577-5581. Massey et al. (1992) *Am. J. Physiol.* 263 (*Cell Physiol.* 32), C494-C501), and  $\alpha$ -linolenic acid (Hasler et al. (1991) *Am. J. Physiol.* 261 (*Cell Physiol.*  
30 30), C161-C168) have been reported to inhibit the gap junction in rat cardiac myocytes, striatal astrocytes, and rat liver epithelial cells, respectively. The limited studies of their structural requirements for gap junction inhibition including the chain length, degree and site of unsaturation, and the

stereochemistry of the double bond(s) roughly follow those detailed herein although fewer fatty acids have been examined in these efforts (Aylsworth et al. (1986) *Cancer Res.* 46, 4527-4533; Spray et al. (1990) *Am. J. Physiol.* 258 (Cell Physiol. 27), C195-205).

The comparisons detailed in Figure 3 suggest that the most effective derivatives of the fatty acids in rat glial cells are the primary amides. However, a wide range of secondary or tertiary amides were also found to also be potent inhibitors of the gap junction-mediated dye transfer. Thus, the hydrogen bond donor capability of the primary amides is not essential to the expression of their properties. Many of these were equipotent with oleamide and the series examined exhibited a clear trend favoring the smaller secondary or tertiary amide substituents. This is evident upon comparing the effects of  $R = \text{NH}_2 > \text{MeNH} > \text{Et}_2\text{N} > \text{c}-(\text{CH}_2)_4\text{N} > \text{PhNH}$ . Although the secondary amides typically were more potent than the corresponding tertiary amides, the comparable potency of the *N*-methyl and *N,N*-dimethyl amide ( $\text{MeNH} = \text{Me}_2\text{N}$ ) is notable. This is consistent with a requirement for small amide substituents and the most potent of the agents examined was the *O*-methyl, *N*-methyl hydroxamide ( $R = \text{MeONMe}$ ). Although the esters of oleic acid were inactive at 50-100  $\mu\text{M}$ , oleyl aldehyde proved to be surprisingly effective while its corresponding dimethyl acetal was inactive. Oleyl aldehyde was found to be nearly equipotent with oleamide and the related agent  $R = \text{CF}_3$  possessing a strongly electrophilic and polarized carbonyl was significantly more potent than oleamide. In contrast, related but less electrophilic ketones ( $R = \text{BrCH}_2 > \text{ClCH}_2$ ) were substantially less effective. Thus, a smooth trend favoring the more polarized or electrophilic carbonyls was observed. The behavior of the former two agents ( $R = \text{H}, \text{CF}_3$ ) is especially significant since they are also potent inhibitors ( $K_i = 190$  and  $1$  nM, respectively) of fatty acid amide hydrolase. Such agents possess dual activities serving both as effective



agonists of oleamide and as potent inhibitors of the enzyme responsible for its degradation. Both exhibit a reversible effect on the gap junction inhibition and removal of the media containing the agents and replacement with fresh media restored  
5 full gap junction function.

Thus, a range of effective although sterically undemanding substitutions can be made for the carboxamide. To date, the effective inhibitors possess a polarized carbonyl at the C-terminus potentially capable of accepting but not necessarily  
10 donating a hydrogen bond.

**Oleyl Ethanolamide, Anandamide and Related Structures.** An important subset of modified carboxamides is the ethanolamide derivatives (Bachur et al. (1965) *J. Biol. Chem.* **240**, 1019-1024; Ramachandran et al. (1992) *Biochem. Arch.* **8**, 369-377; Schmid et  
15 al. (1990) *Prog. Lipid Res.* **29**, 1-43; Hanus et al. (1993) *J. Med. Chem.* **36**, 3032-3034). As discussed *vide supra*, anandamide as well as oleamide were shown to selectively inhibit gap junction communication (dye transfer and electrical coupling) without affecting calcium wave transmission although oleamide  
20 was more potent. This effect in glial cells is distinct from the report that anandamide and oleyl ethanolamide not only inhibit gap junction-mediated dye transfer but also block the propagation of astrocyte calcium waves generated either by mechanical stimulation or local glutamate application (Venance  
25 et al. (1995) *Nature* **376**, 590-594). Consequently, the corresponding ethanolamide of oleic acid and the *bis*-(ethanol)amides of oleic and arachidonic acid were examined as illustrated in Figure 4.

Oleamide proved more potent than oleyl ethanolamide and  
30 discernibly more potent than anandamide at 20  $\mu$ M although both were effective inhibitors at 50  $\mu$ M. Although the effective properties of the oleyl ethanolamide ( $R = \text{HOCH}_2\text{CH}_2\text{NH}$ ) may suggest it represents an important member of another class of active endogenous agents, it is less potent than oleamide, *N*-ethyl

oleamide ( $R = \text{CH}_3\text{CH}_2\text{NH}$ ) which lacks the hydroxyl group, as well as *N*-propyl oleamide ( $R = \text{CH}_3\text{CH}_2\text{CH}_2\text{NH}$ ) which contains a methyl group in place of the hydroxyl group (Figure 3).

In addition, oleyl propanolamide exhibited comparable  
5 properties. Thus, the hydroxyl group of oleyl ethanolamide does not appear to contribute to its gap junction inhibition properties and may actually diminish them. Similar observations were made in the comparison of arachidonyl amide ( $R = \text{NH}_2$ ) and anandamide ( $R = \text{HOCH}_2\text{CH}_2\text{NH}$ ) where the latter was less potent.  
10 Comparable observations have been made in the examination of anandamide analogs ( $R = \text{CH}_3\text{CH}_2\text{CH}_2\text{NH} > \text{HOCH}_2\text{CH}_2\text{NH}$ ) with their binding to the CNS cannabinoid receptor. In contrast, the bis-(ethanol)amides were ineffective at inhibiting the gap junction at  $50 \mu\text{M}$ , and is consistent with their behavior as bulky  
15 tertiary amides which exhibit lower activity.

Recent studies have detailed the isolation of 2-arachidonyl glycerol as a potential endogenous cannabinoid receptor agonist (Mechoulam et al. (1995) *Biochem. Pharmacol.* 50, 83-90) and gap  
20 junction inhibition by diacylglycerols including 1-oleoyl-2-acetylglycerol in Chinese hamster V79 cells has been disclosed (Aylsworth et al. (1986) *Cancer Res.* 46, 4527-4533).

Consequently, we evaluated both racemic and (2*S*)-1-oleoyl-2-acetylglycerol as potential inhibitors. Consistent with the behavior of simple oleic acid esters (Figure 3), both were not  
25 inhibitors of rat glial gap junction-mediated dye transfer.

**Methyl Terminus.** From the studies of the fatty acid primary amides, the length of the fatty acid chain did not substantially reduce the gap junction inhibition properties provided it incorporated the key  $\Delta^9$  cis double bond:  $18:1^9 >$   
30  $20:1^9 = 22:1^9 = 24:1^9$ . This indicates that the methyl terminus can accommodate extensions by a alkyl chain without losing the agonist properties. Similarly, capping the methyl terminus with a methyl ether ( $R = \text{CH}_2\text{OCH}_3$ ) did not effect the gap junction inhibition. In contrast, the introduction of polar



functionality at this site eliminated the inhibitory properties. Thus, the symmetrical 18:1<sup>9</sup> dicarboxamide as well as the terminal alcohol and carboxylic acid exhibited no gap junction inhibition at 50  $\mu$ M, Figure 5.

5        **Putative Precursors.** In view of the possibility that oleamide may be stored as a *N*-oleoyl glycinamide derivative whose formation from oleoyl-CoA is catalyzed by acyl-CoA:glycine *N*-acyltransferase and released upon  $\alpha$ -hydroxylation of glycine by a peptidylglycine  $\alpha$ -amidating monooxygenase (PAM) or a  
10 closely related enzyme, a set of *N*-oleoyl glycine derivatives were examined even before the potential of such a process was experimentally verified (Merkler et al. (1996) *Arch. Biochem. Biophys.* 330, 430-434). Both *N*-oleoyl glycine as well as *N*-oleoyl glycinamide were ineffective as inhibitors of gap  
15 junction-mediated dye transfer, Figure 6. Thus, a putative storage precursor to oleamide as well as *N*-oleoyl glycinamide, which is representative of a peptide or protein terminating with an oleoyl glycinamide, failed to inhibit the gap junction. Interestingly, *N*-oleoyl glycine ethyl ester was an effective,  
20 albeit slightly less potent, inhibitor and most likely represents simply another allowable *N*-substitution on the terminal carboxamide (cf. Figure 3).

In contrast, *N*-oleoyl sarcosine as well as its ethyl ester were found to be effective inhibitors. Initially surprising,  
25 this observation suggests that the intramolecular hydrogen-bonded rotamer of the free acid or the deprotonated carboxylate, which would be observed with *N*-oleoyl glycine but less favored with *N*-oleoyl sarcosine, ties up the amide carbonyl disrupting its intermolecular hydrogen bond acceptor capabilities that may  
30 be required for gap junction inhibition.

**Modifications in the Olefin.** A series of agents 2-8 (Figure 7) were examined to define the role of the olefin and to provide insights into a conformational dependence on activity

(Figure 7). Thus, while the saturated agent octadecanamide and the *trans*-9-octadecenamide were ineffective (cf. Figure 2), 9-octadecynamide (2) was a potent inhibitor being only slightly less active than oleamide. Its conformational characteristics, which more closely resemble those of the inactive 18:0 and 18:19-*trans* than oleamide, suggest that the appropriate presentation of a  $\pi$ -system in addition to the conformation of the *cis* olefin may be important. Consistent with this, the epoxide 3 was inactive and the cyclopropane 4 was less active although both may closely mimic the conformational characteristics of oleamide. Although there are a number of plausible explanations for this, 4 may benefit from the partial  $\pi$  characteristics of the cyclopropane. Alternatively, the incorporation of the epoxide oxygen in 3 may alter the hydrophobic character of the agent and diminish gap junction inhibition. Consistent with either explanation, the agents 5 and 6 proved to be weak inhibitors of the gap junction-mediated dye transfer and 5, which possesses the *cis*  $\Delta^9$  double of oleamide was more potent. The removal of the double bond reduced but did not completely eliminate the inhibition properties suggesting an important role for both the  $\pi$ -character of the double bond as well as its imposed conformational preference for a hairpin conformation. Consistent with this, incorporation of a benzene ring into the structure provided the agents 7-10 that inhibit the gap junction-mediated dye transfer. Importantly, 8 was found to be the most potent in the series being nearly equipotent with oleamide and it most closely mimics the hairpin conformation of oleamide. This was followed closely by 7 which represents an extended version of 8 and oleamide. In contrast, both 9 and 10 were less effective and both mimic alternatives to the hairpin conformation of oleamide.

**Modifications in the Linking Chain and Enzyme Inhibitors: Dual Activities.** In the examination of the fatty acid primary

amides it was established that increasing or shortening the distance between the carboxamide and cis double bond generally diminished the gap junction inhibition. In addition to these studies, other modifications in the 7 carbon chain linking the olefin and carboxamide were also examined, Figure 8. The first set of agents contain modifications that should slow the rate of degradation by enzymatic hydrolysis (Maurelli et al. (1995) *FEBS Lett.* 377, 82-86). Both  $\alpha$ -methyl oleamide and  $\alpha,\alpha$ -dimethyl oleamide were potent inhibitors of the gap junction and both would be expected to be subject to slower FAAH hydrolysis than oleamide. In addition, the agents containing a terminal primary carbamate (X = O) or urethane (X = NH) were also potent inhibitors. The latter is especially interesting since the corresponding urethane of oleyl amine which is two carbons longer in length and whose double bond resides not 9 but 11 carbons from the terminal carbonyl is essentially inactive (Figure 3). Similarly,  $\alpha$ -hydroxy oleamide (X = CHOH),  $\alpha$ -chlorooleamide (X = CHCl) and  $\alpha$ -acetylthiooleamide (X = CHSAC) were effective inhibitors, although the free thiol (X = CHSH) was ineffective. This latter agent suffers from disulfide formation that may account for its ineffectiveness. The last two agents in Figure 8 constitute analogs that are effective ( $K_i$  = 16 and 17 nM, respectively) inhibitors of FAAH. Both serve as oleamide agonists and as inhibitors (Koutek et al. (1994) *J. Biol. Chem.* 269, 22937-22940) of FAAH responsible for its degradation. For both, the gap junction inhibition was reversible and restored to full activity after suspension of the glial cells in fresh media.

30 Example 2: Evidence that Lipid Oleamide Deconvolutes Gap Junction Communication and Calcium Wave Transmission in Glial Cells

Oleamide is a sleep-inducing lipid originally isolated from the cerebrospinal fluid of sleep-deprived cats. Oleamide was

found to potently and selectively inactivate gap junction mediated communication between rat glial cells. In contrast, oleamide had no effect on mechanically-stimulated calcium wave transmission in this same cell type. Other chemical compounds  
5 traditionally employed as inhibitors of gap junctional communication, like heptanol and 18 $\beta$ -glycyrrhetinic acid (18 $\beta$ -GA), blocked not only gap junctional communication, but intercellular calcium signalling as well. Given the central role for intercellular small molecule and electrical signalling  
10 in central nervous system function, oleamide-induced inactivation of glial cell gap junction channels may serve to regulate communication between brain cells, and in doing so, may influence higher order neuronal events like sleep induction.

Studies on the molecular mechanisms for cellular  
15 interactions have traditionally been hindered by a deficiency of natural products that selectively target specific forms of intercellular communication. One primary mode for direct intercellular contact involves the cell-to-cell transmission of molecules through channels in a specialized cell surface  
20 membrane structure, the gap junction (Kumar and Gilula, 1996). Gap junctions allow the passive diffusion of molecules between cells with a selectivity based principally on size, allowing the exclusive movement of molecules smaller than 1000 Da. Such size-selective molecular communication is essential for many  
25 forms of multicellular function, including the regulation of events between cells during embryogenesis and the synchronization of cells in the myocardium (Warner et al., 1984; Dewey and Barr, 1962). Previously, we reported the structure determination of a novel, sleep-inducing lipid, 9(Z)-  
30 octadecenamide, or oleamide, originally isolated from the cerebrospinal fluid of sleep-deprived cats (Cravatt et al., 1995). In our continued efforts to identify and characterize cellular effects associated with oleamide, we now report that oleamide potently and selectively blocks gap junctional

communication in rat glia without altering calcium wave transmission in these cells.

**Results: Gap junction dye coupling and electrical coupling**

5       Gap junction mediated intercellular communication in cultured rat glial cells was evaluated by microinjection and scrape-loading (El-Fouly et al. (1987) *Expl. Cell Res.* 168, 422-430.) of the fluorescent dye, Lucifer yellow. While under control conditions microinjected glial cells demonstrated strong dye coupling as monitored by intercellular Lucifer yellow  
10 diffusion (Figures 10A-10C), pretreatment of these cells with 50  $\mu$ M oleamide for 10 min completely blocked intercellular dye transfer (Figures 10D-10F). Likewise, glial cells scrape-loaded with Lucifer yellow showed significant dye transfer that was  
15 fully abrogated by pretreatment with oleamide (Figures 10G-10I). Dose response and time course studies of oleamide-induced inhibition of dye transfer were conducted (Figures 11J-11K, respectively). Lower doses of oleamide (20  $\mu$ M) also significantly inhibited glial cell dye transfer, but longer  
20 preincubation times (4 h) were required. Oleamide's effects on gap junction permeability proved stable and completely reversible. Thus, whereas no restoration of dye-coupling was observed in glial cells that were continually exposed to oleamide for up to 24 h, once oleamide was removed by changing  
25 the culture media, junctional communication recovered to control levels within 1 h (see Figures 10K, recovery).

As an additional measure of oleamide's effect on gap junction permeability, glial cell gap junctional conductance was examined in the presence of oleamide by using the double whole-  
30 cell recording technique (Figure 12). Consistent with its effect on gap junction-mediated dye transfer, oleamide (50  $\mu$ M) completely blocked junctional electrical coupling in glial cells.



### Structure-activity relationship

To evaluate oleamide's structure-activity relationship, several chemical analogs were synthesized (Cravatt et al. (1996) J. Am. Chem. Soc. 118, 580-590) and tested for their ability to block gap junction permeability (Figure 13). In contrast to oleamide, oleic acid and trans-9-octadecenamide showed no effect on glial cell dye coupling, even at higher doses. Interestingly, oleic acid has previously been demonstrated to inhibit gap junction communication in rat cardiac myocytes, which like rat glia, express the  $\alpha 1$  connexin (Cx43), indicating that the response of the glial cells selectively to oleamide is likely a function of cell type rather than the primary structure of the  $\alpha 1$  connexin. Other *cis*-monounsaturated fatty acid amides, in addition to oleamide, demonstrated varying degrees of inhibition. 50  $\mu$ M *Cis*-11-octadecenamide only slightly affected junctional coupling, but at 100  $\mu$ M levels, the compound completely blocked dye transfer. Oleyl ethanolamide and *cis*-8-octadecenamide were significant inhibitors of dye transfer at 50  $\mu$ M levels, but proved less potent than oleamide at lower doses. Thus, the key chemical features of oleamide that impart upon the compound its inhibitory properties appear to be the amide functionality and the *cis*-double bond, with discernible preference exhibited for a primary amide moiety and location of the degree of unsaturation at the  $\Delta 9$  position along the alkyl chain. Oleamide's effect on glial cells was also compared to the activity of other established inhibitors of gap junction communication. Both 18 $\beta$ -GA and anandamide blocked dye transfer at doses comparable to oleamide, while much higher concentrations of heptanol (3 mM) were required to inhibit junctional communication (Figure 13).

### Oleamide effects on $\beta 1$ connexin-transfected BHK cells

To evaluate whether oleamide's effect on  $\alpha 1$  containing gap junctions was specific to the  $\alpha 1$  junctional type, we determined

dye transfer properties in BHK cells that were transfected with  $\beta 1$  connexin to produce  $\beta 1$  containing gap junctions. For this analysis, the same assay conditions were applied that were used for the rat glial cell measurements. 50  $\mu\text{M}$  Oleamide was found  
5 to rapidly and completely block dye transfer (Figure 14D) between BHK/ $\beta 1$  cells, while oleic acid and trans-9-octadecenamide showed no effect (Figure 14E). Other inhibitory compounds, like anandamide, 18 $\beta$ -GA, and heptanol had inhibitory effects on dye transfer between the BHK/ $\beta 1$  cells that were very  
10 similar to those observed in the rat glial cells (compare Figure 15E and Figure 13).

#### **Gap junctional intercellular communication and the calcium wave**

Since calcium waves in brain cell populations can propagate  
15 in a gap junction-dependent manner, we next evaluated oleamide's effect on calcium wave transmission among glial cells. Using the intracellular calcium indicator, Fluo-3, to monitor changes in intracellular calcium levels within the glial cell population, we found that 50  $\mu\text{M}$  oleamide had no impact on  
20 mechanically-induced calcium wave propagation (Figure 16D-16F and Figure 9). Intrigued by oleamide's contrasting effects on gap junction communication and calcium wave transmission, we compared oleamide's properties to other gap junction inhibitors. 18 $\beta$ -GA (40  $\mu\text{M}$ ) and heptanol (3 mM) showed no discrimination in  
25 their inhibitory activity, completely blocking both dye transfer and calcium wave propagation in glial cells (Figure 16C and Figure 9). In contrast, we found that anandamide, an amidated lipid like oleamide, resembled oleamide in activity, selectively inhibiting gap junction communication (dye transfer and  
30 electrical coupling) without affecting calcium wave transmission (Figure 9).

In mammary gland cells, mast cells, and insulin-secreting cells calcium waves have been shown to propagate by a process dependent on extracellular release of ATP from the stimulated



cell. To examine whether glial cells also transmitted ATP-dependent calcium waves, the glia were treated with the P2-purinergic receptor antagonist, suramin, and subsequently tested for calcium wave transmission. As shown in Figure 9, suramin  
5 (200  $\mu$ M) blocked glial calcium wave transmission without affecting gap junction communication in these cells. Co-treatment of the glia with suramin and oleamide produced the combined phenotype of blocked calcium wave transmission and blocked gap junction communication, further supporting the  
10 notion that the two pathways for these interglial interactions are distinct and separate.

#### Changes of $\alpha$ 1 connexin phosphorylation

In an effort towards defining the molecular mechanism of  
15 oleamide's action on gap junctions, we examined the phosphorylation profile of the  $\alpha$ 1 connexin in glial cells upon treatment with oleamide. The  $\alpha$ 1 connexin has previously been shown by Western blotting to exist in three distinguishable isoforms: nonphosphorylated, NP (~42 kD), and two  
20 phosphorylated isoforms, P1 (~44 kD), and P2 (~46kD). Upon exposure to oleamide (50  $\mu$ M), glial cells demonstrated a dramatic loss of the P2 isoform with no discernible change in the levels of P1 and NP (Figures 17, lane 4). The effect of oleamide on the  $\alpha$ 1 phosphorylation profile proved reversible, as  
25 removal of oleamide from the glial cell culture media was associated with a restoration of P2 to control levels (Figure 17, lane 5). No change in the  $\alpha$ 1 phosphorylation profile was detected in glial cells exposed to oleic acid or trans-9-octadecenamide (Figure 17, lanes 2-3), two oleamide analogs  
30 which did not inhibit gap junction communication.

#### Discussion

The sleep-inducing lipid, oleamide, exhibits the special capacity to block gap junction communication in glial cells as

monitored by dye transfer and electrical conductance without inhibiting intercellular calcium wave transmission in these same cells. Additionally, oleamide induces a dramatic change in the phosphorylation profile of the  $\alpha 1$  connexin protein, the principle component of the glial cell gap junction channels. The loss of both gap junction permeability and  $\alpha 1$  connexin P2 in the presence of oleamide is consistent with previous work indicating that the P2 connexin isoform is associated with the formation of functional gap junction plaques. However, the precise causal relationship between oleamide-induced gap junction blockage and the loss of the connexin phosphorylated P2 isoform remains uncertain, and this must be examined in more detail to determine if there is a specific association between the two events.

The observation that calcium waves can propagate in glial cells when gap junction communication pathways have been eliminated implies that calcium waves in these cells need not, as previously suggested, be exclusively dependent on gap junctional communication. The precise relationship between these observations and previously reported studies on gap junction associated calcium waves remains to be clarified. However, our observation that suramin, a P2- purinergic receptor antagonist, blocked calcium wave transmission in glial cells without affecting gap junction communication suggests that these cells may transmit intercellular calcium signals by an ATP-dependent mechanism akin to those previously reported for mammary gland cells), mast cells, and insulin-secreting cells. Interestingly, the realization that intercellular calcium waves in glial cells can persist without functional gap junctions may help to explain the presence of calcium waves in certain tissues like the retina, where thus far, gap junctional pathways have not been definitively described among all cell types that participate in transmission of the wave).

In the course of studying oleamide's effect on gap junction

communication, we also accumulated evidence that previously identified gap junction inhibitors, such as 18 $\beta$ -GA and heptanol, are not selective in their inhibitory activity on gap junction channels, but rather appear to act as more general nonspecific perturbants of the plasma membrane and its corresponding functions. Since medium chain alcohols and glycerphospholipid derivatives are often used as tools for specifically studying the gap junctions, we would suggest that, in the future, their biological effects be evaluated in the context of the entire cell. Otherwise, the role of gap junctions in complex cellular phenomena like calcium wave transmission may remain obscure.

Although it is not possible yet to determine the precise mechanism that oleamide uses to exert its effect on gap junction channels, the results from this initial analysis indicate that oleamide will block gap junction channels that contain different connexins [ $\alpha$ 1] connexin,  $\beta$ 1 connexin, and a C-terminal truncated mutant of  $\alpha$ 1. Based on these observations, it is reasonable to consider the possibility that oleamide exerts its action on some generalized structural property of the connexin oligomers or channels in the lipid bilayer. Such a mechanism of action would not be dependent on the integrity of the carboxy terminal domain or other diverse primary sequence properties that exist between the members of the connexin multigene family. In this context, oleamide and related molecules such as anandamide, should prove to be very useful reagents, serving as more specific probes for determining the function of gap junction channels *in vivo* than the relatively non-specific reagents that have been previously applied, such as heptanol and glycerphospholipid acid. Furthermore, although oleamide can block gap junction channels that are comprised of different connexins, there appears to be a cell specific property in determining the effect of oleamide on gap junction channels. For example, in a preliminary analysis (Guan et al., unpublished observations), we have observed that the gap junctional communication property between mammalian myocardial

cells is not as sensitive to the inhibitory action of oleamide as the gap junction channels are in other mammalian cell types, a result that is very consistent with a previous report on arachidonimide treatment of cultured rat myo cardial cells.

5 Hence, such cell-specific responses to bioactive lipids, such as oleamide, may be extremely beneficial for protecting the myocardium from the effects of such molecules *in vivo*.

Finally, by blocking gap junction permeability in glial cells, oleamide may be expected to exert intricate modulatory  
10 effects on brain function and physiology, preserving certain glial, and perhaps also glial-neuronal, forms of cell-cell interaction, like calcium wave transmission, in the absence of the chemical and electrical forms of intercellular contact mediated by gap junctions. The precise mechanism by which  
15 oleamide exerts its profound effect on gap junction channels is unknown. The structure of the truncated  $\alpha 1$  connexin channel is altered after treatment with oleamide. Finally, in addition to the potential direct interaction with the assembled gap junction or its associated proteins, a most intriguing possibility is  
20 that oleamide functions by perturbing the lipid environment of membrane proteins and organelles, thus representing a new class of biologically active lipids that act as fluidity transmitters.

#### SYNTHETIC PROTOCOLS

25

**General**  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra were recorded either on a Bruker AM-250, a Bruker AMX-400 or a Bruker AMX-500 spectrometer. Residual protic solvent  $\text{CHCl}_3$  ( $\delta_{\text{H}} = 7.26$  ppm,  $\delta_{\text{C}} = 77.0$ ),  $d_4$ -methanol ( $\delta_{\text{H}} = 3.30$  ppm,  $\delta_{\text{C}} = 49.0$ ) and  $\text{D}_2\text{O}$  ( $\delta_{\text{H}} = 4.80$  ppm,  $\delta_{\text{C}}$  (of  
30  $\text{CH}_3\text{CN}$ ) = 1.7 ppm) or TMS ( $\delta_{\text{H}} = 0.00$  ppm) were used as internal reference. Coupling constants were measured in Hertz (Hz). HRMS were recorded using FAB method in a *m*-nitrobenzylalcohol (NBA) matrix doped with NaI or CsI. Infra-red spectra were recorded on a Perkin-Elmer FTIR 1620 spectrometer. Enantiomeric excess was

determined by HPLC using a Daicel Chemical Industries CHIRALPAK AD column. Optical rotations were measured with an Optical Activity AA-1000 polarimeter. Melting points were taken on a Thomas Hoover capillary melting point apparatus and are  
5 uncorrected. Column chromatography was performed on Merck Kieselgel 60 (230-400 mesh). Analytical thin layer chromatography was performed using pre-coated glass-backed plates (Merck Kieselgel F<sub>254</sub>) and visualized by cerium molybdophosphate or ninhydrin. Diethyl ether, tetrahydrofuran  
10 (THF) and toluene (PhCH<sub>3</sub>) were distilled from sodium-benzophenone ketyl, dichloromethane (DCM) and acetonitrile from calcium hydride. Other solvents and reagents were purified by standard procedures if necessary. TLC was performed on precoated Kieselgel 60 F<sub>254</sub> plates (Merck). Column chromatography was  
15 carried out on Kieselgel 60 (70-230 mesh and 230-400 mesh) and MCI gel CHP-20P (Mitsubishi Chemical, Ind.).

Unless otherwise described, all starting reagents were purchased from Aldrich, Acros, or Sigma.

20 18:0 (stearamide) as illustrated in Figure 2: purchased from Aldrich and recrystallized once before use.

**General procedure for the preparation of fatty amides:** The fatty acid (1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (0.2 M) and  
25 cooled to 0 °C under a N<sub>2</sub> atmosphere. Oxalyl chloride (2M in CH<sub>2</sub>Cl<sub>2</sub>, 3 equiv) was added slowly. The solution was warmed to 25 °C and allowed to stir for 3 h in the dark. The solvent was removed in vacuo and the flask cooled to 0 °C. Excess concentrated NH<sub>4</sub>OH (for 15: ethanolamide was used) was added  
30 slowly and the crude product was purified by chromatography on SiO<sub>2</sub> using EtOAc/hexanes as an eluent. Fatty acids were purchased from Sigma unless otherwise indicated.

14:1' (Myristoleamide) as illustrated in Figure 2 Synthesized



exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography (50%-100% EtOAc/hexanes, gradient elution) to afford 94% desired product: mp 76-78 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 5.29-5.22 (m, 2H), 2.11 (t, 2H, *J* = 7.6 Hz), 1.95 (m, 4H), 1.52 (p, 2H, *J* = 7.3 Hz), 1.25 (m, 12H), 0.83 (m, 3H); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz) δ 179.3, 130.82, 130.77, 36.6, 33.1, 30.8, 30.5, 30.3, 30.2, 28.1, 27.9, 26.9, 23.4, 14.4; IR (NaCl, film)  $\nu_{\max}$  3324, 2923, 2852, 2523, 2466, 2355, 1630, 1526, 1469, 1435, 1410, 1095, 956, 755, 723 cm<sup>-1</sup>; HRFABMS *m/z* (M+H<sup>+</sup>) 226.2164 (requires *m/z* 226.2171).

**16:1<sup>9</sup>(Palmitoleamide)** as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography (50% EtOAc/hexanes) to afford 96% desired product: mp 74-75 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 5.30 - 5.22 (m, 2H), 2.11 (t, 2H, *J* = 7.6 Hz), 1.94 (m, 4H), 1.53 (p, 2H, *J* = 7.1 Hz), 1.25 (m, 16H), 0.82 (t, 3H, *J* = 6.8 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz) δ 179.3, 130.9, 130.8, 36.5, 33.0, 30.8 (2), 30.4, 30.34 (2), 30.27, 30.1, 28.2, 26.8, 23.7, 14.5; IR (NaCl, film)  $\nu_{\max}$  3324, 3003, 2923, 2852, 2523, 2466, 2357, 1631, 1527, 1469, 1437, 1410, 1215, 758 cm<sup>-1</sup>; HRFABMS *m/z* (M+Cs<sup>+</sup>) 386.1448 (requires *m/z* 386.1460).

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**17:1<sup>8</sup>** as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: white solid: mp 68-69 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.64 (br, 1H), 5.43 (br, 1H), 5.38 - 5.26 (m, 2H), 2.19 (t, 2H, *J* = 7.6 Hz), 2.03 - 1.95 (m, 4H), 1.62 (m, 2H), 1.37 - 1.24 (m, 18 H), 0.85 (t, 3H, *J* = 6.9 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 175.6, 130.2, 129.4, 35.9, 31.9, 29.7, 29.5, 29.4, 29.3, 28.8, 27.2, 27.0, 25.4, 22.7, 14.1; IR (film)

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$\nu_{\max}$  3359, 3193, 2922, 2852, 1659, 1633, 1466, 1412, 1136  $\text{cm}^{-1}$ ;  
FABHRMS (NBA-CsI)  $m/z$  400.1603 ( $\text{C}_{17}\text{H}_{33}\text{NO} + \text{Cs}^+$  requires 400.1616).  
8Z-heptadecenoic acid prepared as previously described (JACS,  
1996, 118, 5938-5945).

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**18:1' (Petroselinamide)** as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography:

10 white solid: mp 73-74 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.15 (br, 1H), 5.61 (br, 1H), 5.34 - 5.27 (m, 2H), 2.17 (t, 2H,  $J = 7.5$  Hz), 2.01 (q, 2H,  $J = 7.0$  Hz), 1.96 (q, 2H,  $J = 7.0$  Hz), 1.60 (p, 2H,  $J = 7.8$  Hz), 1.35 (p, 2H,  $J = 7.8$  Hz), 1.28 - 1.21 (m, 18H), 0.83 (t, 3H,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.9,  
15 130.4, 129.0, 35.8, 31.9, 29.7, 29.62, 29.59, 29.5, 29.3, 29.2, 27.2, 26.8, 25.1, 22.6, 14.1; IR (film)  $\nu_{\max}$  3366, 3203, 3000, 2917, 2848, 1647, 1465, 1415, 734  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  282.2810 ( $\text{C}_{18}\text{H}_{35}\text{NO} + \text{H}^+$  requires 282.2797).

20 **18:1'** as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: white solid: mp 70-71 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.26 (br, 1H), 5.67 (br, 1H), 5.34 -  
25 5.24 (m, 2H), 2.16 (t, 2H,  $J = 7.6$  Hz), 2.00 - 1.92 (m, 4H), 1.58 (p, 2H,  $J = 7.5$  Hz), 1.31 - 1.21 (m, 20H), 0.83 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  176.1, 130.1, 129.3, 35.9, 31.8, 29.7, 29.6 (2), 29.5, 29.4, 29.3, 29.2, 28.8, 27.1, 27.0, 25.4, 22.6, 14.0; IR (film)  $\nu_{\max}$  3392, 3179, 3013, 2920, 2848,  
30 1647, 1468, 1416, 1312, 1115, 806, 719, 628  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  282.2801 ( $\text{C}_{18}\text{H}_{35}\text{NO} + \text{H}^+$  requires 282.2797).

**18:1'** as illustrated in Figure 2 Synthesized exactly as found in



the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography or prepared as previously described exactly (JACS, 1996, 118, 580-590).

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18:1<sup>9</sup>(Oleamide) as illustrated in Figure 2: Prepared as previously described exactly (JACS, 1996, 118, 580-590).

18:1<sup>9trans</sup>(Elaidamide) as illustrated in Figure 2: Prepared as previously described. (JACS, 1996, 118, 580-590).

18:1<sup>11</sup>(Vaccenamide) as illustrated in Figure 2: Prepared as previously described. (JACS, 1996, 118, 580-590).

15 18:1<sup>12</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: white solid: mp 77-78 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 6.11 (br, 1H), 5.62 (br, 1H), 5.34 - 5.26 (m, 2H), 2.16 (t, 2H, *J* = 7.5 Hz), 1.97 (q, 4H, *J* = 6.0 Hz), 1.58 (p, 2H, *J* = 7.3 Hz), 1.32 - 1.22 (m, 20H), 0.84 (t, 3H, *J* = 7.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 176.1, 129.84, 129.78, 35.9, 31.5, 29.7, 29.51, 29.46, 29.42, 29.38, 29.3, 29.23, 29.18, 27.1, 25.5, 22.5, 14.0; IR (film) *ν*<sub>max</sub> 3356, 3188, 2917, 2848, 1727, 1661, 1633, 1469, 1410, 1135, 700, 628 cm<sup>-1</sup>; FABHRMS (NBA-CsI) *m/z* 282.2810 (C<sub>18</sub>H<sub>35</sub>NO + H<sup>+</sup> requires 282.2797).

18:1<sup>13</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: white solid: mp 86-87 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.03 (br, 1H), 5.57 (br, 1H), 5.34 - 5.27 (m, 2H), 2.17 (t, 2H, *J* = 7.6 Hz), 1.98 (m, 4H), 1.58

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(p, 2H,  $J = 7.2$  Hz), 1.30 - 1.22 (m, 20H), 0.86 (t, 3H,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  176.0, 129.82, 129.78, 35.9, 31.9, 29.7, 29.55 (2), 29.48, 29.4, 29.3, 29.24, 29.19, 27.2, 26.8, 25.5, 22.3, 13.9; IR (film)  $\nu_{\text{max}}$  3357, 3192, 3003, 2917, 2848, 1656, 1632, 1470, 1422, 1410, 1136, 721, 636  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  304.2608 ( $\text{C}_{18}\text{H}_{35}\text{NO} + \text{Na}^+$  requires 304.2616).

18:1<sup>15</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: white solid: mp 92-93 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  5.70 (br, 1H), 5.46 (br, 1H), 5.35 - 5.27 (m, 2H), 2.19 (t, 2H,  $J = 7.5$  Hz), 1.99 (h, 4H,  $J = 6.8$  Hz), 1.60 (p, 2H,  $J = 7.3$  Hz), 1.31 - 1.22 (m, 20H), 0.92 (t, 3H,  $J = 7.5$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.7, 131.5, 129.3, 35.9, 29.7, 29.60, 29.56, 29.5, 29.4, 29.31, 29.26, 29.2, 27.1, 25.5, 20.5, 14.4; IR (film)  $\nu_{\text{max}}$  3356, 3189, 2918, 2848, 1659, 1632, 1470, 1420, 1410, 1137  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  282.2707 ( $\text{C}_{18}\text{H}_{35}\text{NO} + \text{H}^+$  requires 282.2797).

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19:1<sup>10</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: white solid: mp 71-72 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.95 (br, 1H), 5.53 (br, 1H), 5.35 - 5.26 (m, 2H), 2.17 (t, 2H,  $J = 7.6$  Hz), 1.97 (m, 4H), 1.59 (p, 2H,  $J = 7.2$  Hz), 1.25 - 1.23 (m, 22H), 0.84 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  176.0, 129.9, 129.7, 35.9, 31.9, 29.7, 29.5, 29.34, 29.27, 29.2, 27.2, 25.5, 22.6, 14.1; IR (film)  $\nu_{\text{max}}$  3362, 3194, 2920, 2850, 1651, 1633, 1469, 1422  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  296.2961 ( $\text{C}_{19}\text{H}_{37}\text{NO} + \text{H}^+$  requires 296.2953). 10Z-nonadecenoic acid prepared as previously described (JACS, 1996, 118, 5938-5945).

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20:1<sup>5</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: (50%-100% EtOAc/hexanes, gradient elution) to afford 86% desired product; mp 82 °C.; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 5.35-5.23 (m, 2H), 2.11 (t, 2H, J = 7.6 Hz), 2.02-1.93 (m, 4H), 1.56 (p, 2H, J = 7.5 Hz), 1.28 - 1.15 (m, 24H), 0.81 (t, 3H, J = 6.9 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 175.5, 131.2, 128.4, 35.2, 31.9, 29.7, 29.7, 29.67, 29.63, 29.3, 27.3, 26.5, 25.4, 22.7, 14.1; IR (NaCl, film)  $\nu_{\max}$  3371, 2917, 2849, 2535, 2361, 1632, 1511, 1471, 1423 cm<sup>-1</sup>; HRFABMS m/z (M+H<sup>+</sup>) 310.3100 (requires m/z 310.3110)

20:1<sup>8</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: white solid: mp 71-72 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 5.70 (br, 1H), 5.44 (br, 1H), 5.36 - 5.27 (m, 2H), 2.19 (t, 2H, J = 7.5 Hz), 1.99 - 1.96 (m, 4H), 1.61 (p, 2H, J = 7.0 Hz), 1.30 - 1.23 (m, 24H), 0.85 (t, 3H, J = 7.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 175.7, 130.1, 129.6, 35.9, 31.9, 29.73, 29.66, 29.62, 29.54, 29.33, 29.31, 29.1, 29.0, 27.2, 27.1, 25.5, 22.7, 14.1; IR (film)  $\nu_{\max}$  3389, 3200, 3003, 2917, 2848, 1645, 1467, 1418, 722 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 310.3125 (C<sub>20</sub>H<sub>39</sub>NO + H<sup>+</sup> requires 310.3110).

20:1<sup>9</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: The crude product was chromatographed (SiO<sub>2</sub>, 1 x 10 cm, 50 - 100% EtOAc/hexanes, gradient elution) to afford 9 (21.4 mg, 61%) as a white solid: mp 72-73 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.56 (br, 1H), 5.41 (br,

1H), 5.36 - 5.27 (m, 2H), 2.19 (t, 2H,  $J = 7.6$  Hz), 1.99 - 1.96 (m, 4H), 1.61 (p, 2H,  $J = 7.1$  Hz), 1.28 - 1.23 (m, 28H), 0.85 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.6, 130.0, 129.7, 35.9, 31.9, 29.75, 29.67, 29.64, 29.55, 29.34, 29.31, 29.22, 29.19, 29.10, 27.2, 27.1, 25.5, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  3390, 3199, 2917, 2848, 1643, 1467, 722  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  338.3416 ( $\text{C}_{22}\text{H}_{43}\text{NO} + \text{H}^+$  requires 338.3423). Acid prepared in a manner analogous to 22:1<sup>9</sup>.

10 20:1<sup>11</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: White solid was purified by chromatography (50%-66% EtOAc/hexanes, gradient elution) to afford 97% desired product; mp 81-82 °C;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  5.29 - 5.21 (m, 2H), 2.10 (t, 2H,  $J = 7.6$  Hz), 1.93 (m, 4H), 1.51 (p, 2H,  $J = 7.3$  Hz), 1.22 (m, 24H), 0.83 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  179.3, 130.8 (2), 36.6, 33.1, 30.88, 30.86, 30.6, 30.5, 30.4, 28.2, 28.1, 26.9, 23.8, 14.5; IR (NaCl, film)  $\nu_{\text{max}}$  3324, 2918, 2849, 2522, 2353, 1629, 1527, 1468, 1437, 1410, 955, 721  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $\text{M} + \text{Cs}^+$ ) 442.2068 (requires  $m/z$  442.2086).

20:1<sup>13</sup> as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: White solid was purified by chromatography (33% EtOAc/hexanes) to afford 89% desired product; mp 84-85 °C;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  5.29 - 5.21 (m, 2H), 2.10 (t, 2H,  $J = 7.6$  Hz), 1.93 (m, 4H), 1.51 (p, 2H,  $J = 7.2$  Hz), 1.21 (m, 24H), 0.81 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  179.3, 130.9 (2), 36.6, 33.0, 30.9 (2), 30.8 (2), 30.7 (2), 30.5, 30.3 (2), 30.1, 28.2, 28.1, 26.9, 23.8, 14.5;

IR (NaCl, film)  $\nu_{\max}$  3324, 2917, 2849, 2523, 2347, 1630, 1526, 1470, 1430, 1410, 953, 721  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $M+H^+$ ) 310.3099 (requires  $m/z$  310.3110).

5 22:1' as illustrated in Figure 2 and route illustrated in Figure 18: 7-bromoheptanol was protected (TBDPSCl, Et<sub>3</sub>N, DMAP) and treated with PPh<sub>3</sub> to generate a phosphonium salt (284.9 mg, 0.42 mmol, 1 eq) that was dissolved in anhydrous THF (2.6 mL) under Ar at -78 °C was treated dropwise with KHMDS (0.5M in toluene, 10 0.85 mL, 0.43 mmol, 1 eq). The orange solution was allowed to stir for 40 min at -78 °C before tridecanal (100  $\mu$ L, 0.42 mmol, 1 eq) was added. The reaction was warmed to 25 °C and allowed to stir 1 h before saturated aqueous NH<sub>4</sub>Cl (30 mL) was added. The aqueous layer was extracted with EtOAc (3 x 30 mL). The organic 15 layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 2 x 8 cm, 0 - 5% EtOAc/hexanes, gradient elution) to afford **alkene** (131.0 mg, 55%) as a clear oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  7.69 (dd, 4H,  $J$  = 8.0, 1.5 Hz), 7.44 - 7.37 (m, 6H), 5.40 - 5.34 (m, 2H), 3.67 (t, 20 2H,  $J$  = 6.5 Hz), 2.03 (q, 4H,  $J$  = 6.0 Hz), 1.58 (p, 2H,  $J$  = 7.5 Hz), 1.35 - 1.28 (m, 30H), 1.07 (s, 9H), 0.90 (t, 3H,  $J$  = 7.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  135.6, 134.2, 129.90, 129.86, 129.5, 127.5, 64.0, 32.6, 31.9, 29.8, 29.70, 29.67, 29.6, 29.5, 29.4, 29.33, 29.26, 27.2, 26.9, 25.8, 22.7, 19.2, 14.1; IR 25 (film)  $\nu_{\max}$  2925, 2854, 1472, 1457, 1112, 823, 701, 608  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  695.3649 (C<sub>38</sub>H<sub>62</sub>OSi + Cs<sup>+</sup> requires 695.3624).

A solution of **alkene** (128.2 mg, 0.23 mmol, 1 eq) in THF (2.2 mL) under N<sub>2</sub> was treated with TBAF (1M in THF, 0.46 mL, 0.46 30 mmol, 2 eq) and stirred at 25 °C for 2.5 h. Water (30 mL) was added and the aqueous layers were extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 2 x 5 cm, 5 - 33% EtOAc/hexanes, gradient elution) to

afford **alcohol** (51.2 mg, 69%) as a white film;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.36 - 5.29 (m, 2H), 3.62 (t, 2H,  $J = 6.6$  Hz), 2.02 - 1.97 (m, 4H), 1.54 (p, 2H,  $J = 6.8$  Hz), 1.28 - 1.24 (m, 30H), 0.86 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  129.9, 129.8, 63.1, 32.8, 31.9, 29.73, 29.67, 29.64, 29.6, 29.5, 29.39, 29.35, 29.31, 29.2, 27.2, 25.7, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  3328, 2923, 2852, 1457, 1055  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  325.3475 ( $\text{C}_{22}\text{H}_{44}\text{O} + \text{H}^+$  requires 325.3470).

A solution of **alcohol** (48.5 mg, 0.15 mmol, 1 eq) in anhydrous DMF (1 mL) under  $\text{N}_2$  was treated with PDC (0.28 g, 0.74 mmol, 5 eq) and stirred at 25  $^\circ\text{C}$  for 3.5 h. The reaction mixture was filtered through celite and the filtrate was concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 1 x 8 cm, 10 - 20% EtOAc/hexanes, gradient elution) to afford **acid** (36.2 mg, 72%) as a clear oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.37 - 5.28 (m, 2H), 2.33 (t, 2H,  $J = 7.5$  Hz), 2.01 - 1.97 (m, 4H), 1.61 (p, 2H,  $J = 7.2$  Hz), 1.29 - 1.24 (m, 28H), 0.86 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  179.9, 130.0, 129.7, 34.0, 31.9, 29.8, 29.7, 29.6, 29.4, 29.3, 29.14, 29.06, 29.03, 27.2, 27.1, 24.7, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  2918, 2850, 1689, 1466, 1411, 1297, 918, 722, 687  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  361.3091 ( $\text{C}_{22}\text{H}_{42}\text{O}_2 + \text{Na}^+$  requires 361.3083).

A solution of **acid** (34.9 mg, 0.103 mmol, 1 eq) in anhydrous  $\text{CH}_2\text{Cl}_2$  (0.5 mL) under  $\text{N}_2$  at 0  $^\circ\text{C}$  was treated with oxalyl chloride (2M in  $\text{CH}_2\text{Cl}_2$ , 0.13 mL, 0.26 mmol, 3 eq). The reaction mixture was warmed to 25  $^\circ\text{C}$  and stirred for 3 h before the solvent was removed in vacuo. The residue was cooled to 0  $^\circ\text{C}$  and treated with excess concentrated  $\text{NH}_4\text{OH}$  (1 mL). Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 1 x 10 cm, 50 - 100% EtOAc/hexanes, gradient elution) to afford **22:1'** (21.4 mg, 61%) as a white solid: mp 72-73  $^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)



$\delta$  5.56 (br, 1H), 5.41 (br, 1H), 5.36 - 5.27 (m, 2H), 2.19 (t, 2H,  $J = 7.6$  Hz), 1.99 - 1.96 (m, 4H), 1.61 (p, 2H,  $J = 7.1$  Hz), 1.28 - 1.23 (m, 28H), 0.85 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.6, 130.0, 129.7, 35.9, 31.9, 29.75, 29.67, 29.64, 29.55, 29.34, 29.31, 29.22, 29.19, 29.10, 27.2, 27.1, 25.5, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  3390, 3199, 2917, 2848, 1643, 1467, 722  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  338.3416 ( $\text{C}_{22}\text{H}_{43}\text{NO} + \text{H}^+$  requires 338.3423).

10 **22:1<sup>13</sup>(Erucamide)** as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: White solid was purified by chromatography (50% EtOAc/hexanes) to  
15 afford 96% desired product; mp 83-84 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.29 - 5.21 (m, 2H), 2.09 (t, 2H,  $J = 7.6$  Hz), 1.93 (m, 4H), 1.51 (p, 2H,  $J = 7.3$  Hz), 1.20 (m, 28H), 0.81 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.8, 129.88, 129.85, 36.0, 31.9, 29.7, 29.58, 29.53, 29.50, 29.46, 29.32, 29.29, 29.18,  
20 27.2, 25.3, 22.7, 14.1; IR (NaCl, film)  $\nu_{\text{max}}$  3375, 2920, 2847, 2544, 1633, 1511, 1468, 1417, 932, 721  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $\text{M}+\text{Cs}^+$ ) 470.2413 (requires  $m/z$  470.2399).

**24:1'** as illustrated in Figure 2 Synthesized exactly as found in  
25 the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: ( $\text{SiO}_2$ , 1 x 10 cm, 50 - 100% EtOAc/hexanes, gradient elution) to afford 13 (20.7 mg, 67%) as a white solid: mp 74-75 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  5.49 (br,  
30 1H), 5.39 (br, 1H), 5.35 - 5.28 (m, 2H), 2.19 (t, 2H,  $J = 7.8$  Hz), 2.00 - 1.96 (m, 4H), 1.61 (p, 2H,  $J = 7.0$  Hz), 1.29 - 1.23 (m, 32H), 0.85 (t, 3H,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.5, 130.0, 129.7, 35.9, 31.9, 29.8, 29.68, 29.65, 29.56,

29.3, 29.23, 29.19, 29.1, 27.21, 27.15, 25.5, 22.7, 14.1; IR (film)  $\nu_{\max}$  3389, 3184, 2917, 2848, 1044, 1467, 1416, 1119  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  366.3740 ( $\text{C}_{24}\text{H}_{47}\text{NO} + \text{H}^+$  requires 366.3736). Acid prepared in a manner analogous to 22:1<sup>9</sup>.

5

**24:1<sup>15</sup>(Nervonamide)** as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: White  
10 solid was purified by chromatography (33%-66% EtOAc/hexanes, gradient elution) to afford 91% desired product; mp 87-88 °C; <sup>1</sup>H NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.36-5.28 (m, 2H), 2.19 (t, 2H,  $J = 7.6$  Hz), 1.98 (m, 4H), 1.60 (p, 2H,  $J = 7.2$  Hz), 1.36-1.23 (m, 32H), 0.85 (t, 3H,  $J = 6.8$  Hz); <sup>13</sup>C NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.7,  
15 129.9 (2), 35.94, 35.91, 31.9, 29.8, 29.63, 29.56, 29.54, 29.50, 29.46, 29.33, 29.30, 29.22, 27.18, 25.5, 22.7, 14.1; IR (NaCl, film)  $\nu_{\max}$  3392, 2919, 2847, 2543, 2358, 1633, 1468, 1412, 720  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $\text{M}+\text{H}^+$ ) 366.3727 (requires  $m/z$  366.3736).

20 **18:2<sup>9,12</sup>(Linoleamide)** as illustrated in Figure 2: Prepared as previously described. (JACS, 1996, 118, 580-590).

**18:2<sup>9,12-trans</sup>(Linoelaidamide)** as illustrated in Figure 2  
Synthesized exactly as found in the general procedure *vide supra*;  
25 *supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting white solid was purified by chromatography: (50% EtOAc/hexanes) to afford 88% desired product; mp 83-84 °C.; <sup>1</sup>H NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  5.35 - 5.24 (m, 4H), 2.59 (m, 2H), 2.10 (t, 2H,  $J = 7.6$  Hz), 1.90 (m, 4H), 1.51  
30 (p, 2H,  $J = 7.2$  Hz), 1.33 - 1.13 (m, 14H), 0.81 (t, 3H,  $J = 7.0$  Hz); <sup>13</sup>C NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  179.3, 131.99, 131.92, 130.0, 129.9, 36.6, 36.5, 33.6 (2), 32.5, 30.7, 30.4, 30.3 (2), 30.1, 26.9, 23.6, 14.5; IR (NaCl, film)  $\nu_{\max}$  3371, 2917, 2847, 2540,

2345, 1622, 1470, 1420, 964, 716  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $M+\text{Cs}^+$ )  
412.1598 (requires  $m/z$  412.1616)

20:2<sup>11,14</sup> as illustrated in Figure 2 Synthesized exactly as found  
5 in the general procedure *vide supra*; all starting reagents were  
purchased from Aldrich, Acros, or Sigma and resulting white  
solid was purified by chromatography: (50%-100% EtOAc/hexanes,  
gradient elution) to afford 92% desired product; mp 47-49 °C; <sup>1</sup>H  
NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  5.31-5.20 (m, 4H), 2.67 (t, 2H,  $J$  = 6.2  
10 Hz), 2.12 (t, 2H,  $J$  = 7.6 Hz), 1.97 (q, 4H,  $J$  = 6.8 Hz), 1.52  
(p, 2H,  $J$  = 7.3 Hz), 1.32 - 1.17 (m, 18H), 0.82 (t, 3H,  $J$  =  
6.9); <sup>13</sup>C NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  179.2, 130.9 (2), 129.1 (2),  
36.5, 32.7, 30.8 (2), 30.7 (2), 30.5 (2), 30.41, 30.36, 28.2,  
26.9, 26.6, 23.7, 14.5; IR (NaCl, film)  $\nu_{\text{max}}$  3324, 3009, 2919,  
15 2849, 2513, 2466, 2400, 1733, 1634, 1528, 1469, 1410, 1250,  
1048, 758  $\text{cm}^{-1}$ ; LRFABMS  $m/z$  ( $M+\text{H}^+$ ) 308.

18:3<sup>9,12,15</sup>: ( $\alpha$ -linolenamide) as illustrated in Figure 2  
Synthesized exactly as found in the general procedure *vide supra*  
20 *supra*; all starting reagents were purchased from Aldrich, Acros,  
or Sigma and resulting yellow gel was purified by  
chromatography: (33%-100% EtOAc/hexanes, gradient elution) to  
afford 99% desired product. <sup>1</sup>H NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  5.33-5.18  
(m, 6H), 2.76 (t, 4H,  $J$  = 6.0 Hz), 2.11 (t, 2H,  $J$  = 7.6 Hz),  
25 2.00 (m, 4H), 1.52 (p, 2H,  $J$  = 7.1 Hz), 1.26 (m, 8H), 0.89 (t,  
3H,  $J$  = 7.5 Hz); <sup>13</sup>C NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  179.2, 132.7, 131.1,  
129.2 (2), 128.8, 128.2, 36.5, 30.7, 30.4, 30.31, 30.26, 28.2,  
26.9, 26.5, 26.4, 21.5, 14.7; IR (NaCl, film)  $\nu_{\text{max}}$  3326, 3009,  
2924, 2851, 2526, 2356, 1629, 1527, 1469, 1436, 1410, 955, 723  
30  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $M+\text{Cs}^+$ ) 410.1452 (requires  $m/z$  410.1460).

20:3<sup>8,11,14</sup> as illustrated in Figure 2 Synthesized exactly as found  
in the general procedure *vide supra*; all starting reagents were

purchased from Aldrich, Acros, or Sigma and resulting yellow gel was purified by chromatography: (50%-100% EtOAc/hexanes, gradient elution) to afford 78% desired product.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  5.32-5.21 (m, 6H), 2.73 (m, 4H), 2.11 (t, 2H,  $J = 7.6$  Hz), 1.98 (m, 4H), 1.51 (p, 2H,  $J = 7.2$  Hz), 1.34-1.20 (m, 12H), 0.81 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  179.3, 131.1, 131.0, 129.22, 129.16, 128.9, 128.8, 36.5, 32.7 30.7, 30.5, 30.3, 30.1, 28.2 (2), 26.9, 26.6 (2), 23.7, 14.5; IR (NaCl, film)  $\nu_{\text{max}}$  3328, 3009, 2925, 2852, 2525, 2468, 2402, 1634, 1527, 1466, 1411, 1330, 1202, 959  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $\text{M}+\text{Cs}^+$ ) 438.1756 (requires  $m/z$  438.1773).

**20:3<sup>11,14,17</sup>** as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and resulting yellow gel was purified by chromatography: (50%-100% EtOAc/hexanes, gradient elution) to afford 84% desired product.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  5.32-5.17 (m, 6H), 2.71 (t, 4H,  $J = 5.9$  Hz), 2.10 (t, 2H,  $J = 7.6$  Hz), 2.01-1.92 (m, 4H), 1.51 (p, 2H,  $J = 7.3$  Hz), 1.29 - 1.23 (m, 12H), 0.88 (t, 3H,  $J = 7.6$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  179.3, 132.7, 131.1, 129.21, 129.17, 128.8, 128.2, 36.5, 30.8, 30.64, 30.62, 30.5, 30.4, 30.3, 28.2, 26.9, 26.5, 26.4, 21.5, 14.7; IR (NaCl, film)  $\nu_{\text{max}}$  3324, 3010, 2963, 2919, 2849, 2525, 2351, 1627, 1529, 1469, 1439, 1410, 956, 721  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $\text{M}+\text{Cs}^+$ ) 438.1765 (requires  $m/z$  438.1773).

**20:4<sup>5,8,11,14</sup>** as illustrated in Figure 2: purchased from Sigma.

**20:5<sup>5,8,11,14,17</sup>** as illustrated in Figure 2 Synthesized exactly as found in the general procedure *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma and clear oil gel was purified by chromatography:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  5.38 -

5.35 (m, 10H), 2.83 - 2.78 (m, 8H), 2.21 (t, 2H,  $J = 7.5$  Hz),  
2.11 (q, 2H,  $J = 6.5$  Hz), 2.06 (p, 2H,  $J = 7.5$  Hz), 1.71 (p, 2H,  
 $J = 7.5$  Hz), 0.95 (t, 3H,  $J = 7.5$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$   
175.1, 132.1, 129.0, 128.9, 128.6, 128.3, 128.19, 128.16,  
128.07, 127.8, 35.1, 26.5, 25.6, 25.5, 25.2, 20.6, 14.3; IR  
(film)  $\nu_{\text{max}}$  3354, 3192, 3011, 2961, 1660, 1614, 1441, 1410, 1264  
 $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  302.2489 ( $\text{C}_{20}\text{H}_{31}\text{NO} + \text{H}^+$  requires  
302.2484).

10 **22:6<sup>4,7,10,13,16,19</sup>** as illustrated in Figure 2 Synthesized exactly as  
found in the general procedure *vide supra*; all starting reagents  
were purchased from Aldrich, Acros, or Sigma and clear oil gel  
was purified by chromatography: (50%-100% EtOAc/hexanes,  
gradient elution) to afford 55% desired product.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ,  
15 400 MHz)  $\delta$  5.33 - 5.17 (m, 12H), 2.80 - 2.71 (m, 10H), 2.31 (m,  
2H), 2.14 (t, 2H,  $J = 7.4$  Hz), 1.98 (p, 2H,  $J = 7.4$  Hz), 0.87  
(t, 3H,  $J = 7.6$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  178.4, 132.8,  
130.1, 129.4, 129.23, 129.19, 129.16, 129.09, 128.9, 128.2,  
36.3, 26.54, 26.49, 26.42, 24.5, 21.5, 14.7; IR (NaCl, film)  
20  $\nu_{\text{max}}$  3341, 3198, 3012, 2955, 1661, 1393, 1262, 923  $\text{cm}^{-1}$ ; HRFABMS  
 $m/z$  ( $\text{M}+\text{Cs}^+$ ) 460.1640 (requires  $m/z$  460.1616).

**General procedure for the preparation of oleic amide derivatives  
varying about carboxamide (unless otherwise described) as**  
25 **illustrated in Figure 3:** One equivalent of oleic acid was  
dissolved in dry  $\text{CH}_2\text{Cl}_2$  (0.2 M) and cooled to 0 °C under a  $\text{N}_2$   
atmosphere. Oxalyl chloride (2M in  $\text{CH}_2\text{Cl}_2$ , 3 equiv) was added  
slowly. The solution was warmed to 25 °C and allowed to stir  
for 3 hours in the dark. The solvent was then removed in vacuo  
30 and the flask cooled to 0 °C. Excess free amine (amines that  
were available as the hydrochloride salts were extracted into  
EtOAc from a 50% NaOH solution before use) or alcohols were  
added slowly. The crude product was purified by chromatography  
on  $\text{SiO}_2$  using EtOAc/hexanes as an eluent.

Oleic acid: Agent purchased from Aldrich.

MeNH as illustrated in Figure 3 Synthesized exactly as found in the general procedure for derivatives varying about carboxamide  
5 vida supra; all starting reagents were purchased from Aldrich, Acros, or Sigma: White solid was purified by chromatography (50% EtOAc/hexanes) to afford 95% desired product; mp 34-35 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 5.29 - 5.21 (m, 2H), 2.61 (s, 3H), 2.07 (t, 2H, J = 7.6 Hz), 1.93 (m, 4H), 1.50 (p, 2H, J = 7.0 Hz),  
10 1.23 - 1.21 (m, 20H), 0.81 (t, 3H, J = 6.9 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz) δ 176.7, 130.9, 130.8, 37.0, 33.1, 30.87, 30.85, 30.7, 30.5, 30.4, 30.34, 30.27, 28.2, 27.0, 26.3, 23.8, 14.5; IR (NaCl, film) ν<sub>max</sub> 3301, 3005, 2921, 2853, 2418, 1651, 1557, 1463, 1403, 1164, 723 cm<sup>-1</sup>; HRFABMS m/z (M+H<sup>+</sup>) 296.2940 (requires m/z  
15 296.2953).

Me<sub>2</sub>N as illustrated in Figure 3 Synthesized exactly as found in the general procedure for derivatives varying about carboxamide  
vida supra; all starting reagents were purchased from Aldrich,  
20 Acros, or Sigma: Pale yellow oil was purified by chromatography (33% EtOAc/hexanes) to afford 99% desired product. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 5.32 - 5.24 (m, 2H), 2.99 (s, 3H), 2.86 (s, 3H), 2.29 (t, 2H, J = 7.6 Hz), 1.97 (m, 4H), 1.53 (p, 2H, J = 7.3 Hz), 1.28 - 1.24 (m, 20H), 0.85 (t, 3H, J = 6.8 Hz); <sup>13</sup>C NMR  
25 (CD<sub>3</sub>OD, 100 MHz) δ 175.4, 130.82, 130.77, 37.8, 35.7, 34.2, 33.1, 30.90, 30.87, 30.7, 30.5, 30.4, 30.3, 28.2, 26.3, 23.8, 14.6; IR (NaCl, film) ν<sub>max</sub> 2923, 2853, 1652, 1463, 1394, 1267, 1141, 723 cm<sup>-1</sup>; HRFABMS m/z (M+H<sup>+</sup>) 310.3101 (requires m/z 310.3110).

30 EtNH derivative as illustrated in Figure 3 Synthesized exactly as found in the general procedure for derivatives varying about carboxamide vida supra; all starting reagents were purchased from Aldrich, Acros, or Sigma: White solid was purified by



chromatography (33% EtOAc/hexanes) to afford 99% desired product; mp 34-35 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.53 (br, 1H), 5.35 - 5.26 (m, 2H), 3.28 - 3.21 (m, 2H), 2.11 (t, 2H, J = 7.7 Hz), 2.01 - 1.92 (m, 4H), 1.58 (p, 2H, J = 7.4 Hz), 1.30 - 1.23 (m, 20H), 1.09 (t, 3H, J = 7.3 Hz), 0.83 (t, 3H, J = 6.9 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 173.0, 130.0, 129.7, 36.8, 34.2, 31.9, 29.73, 29.67, 29.5, 29.29 (2), 29.25 (2), 29.1, 27.2, 27.1, 25.8, 22.7, 14.9, 14.1; IR (NaCl, film)  $\nu_{\max}$  3304, 3084, 2918, 2850, 1641, 1551, 1466, 938, 721 cm<sup>-1</sup>; HRFABMS m/z (M+H<sup>+</sup>) 310.3099 (requires m/z 310.3110).

**Et<sub>2</sub>N derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma: Pale orange oil was purified by chromatography (20%-50% EtOAc/hexanes, gradient elution) to afford 99% desired product. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 5.32 - 5.25 (m, 2H), 3.31 (p, 4H, J = 7.2 Hz), 2.28 (t, 2H, J = 7.6 Hz), 1.97 (m, 4H), 1.55 (p, 2H, J = 7.3 Hz), 1.29-1.24 (m, 20H), 1.13 (t, 3H, J = 7.1 Hz), 1.04 (t, 3H, J = 7.1 Hz), 0.85 (t, 3H, J = 6.9 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz) δ 174.4, 130.81, 130.77, 43.4, 41.4, 33.9, 33.1, 30.91, 30.87, 30.7, 30.5, 30.4, 30.3, 28.3, 26.7, 23.8, 14.7, 14.6, 13.4; IR (NaCl, film)  $\nu_{\max}$  2925, 2853, 1651, 1462, 1427, 1379, 1309, 1261, 1223, 1139, 1096, 944, 791, 722 cm<sup>-1</sup>; HRFABMS m/z (M+H<sup>+</sup>) 338.3430 (requires m/z 338.3423).

**PrNH derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma: white solid: mp 28-29 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.01 (br, 1H), 5.29 - 5.21 (m, 2H), 3.12 (q, 2H, J = 6.8 Hz), 2.08 (t, 2H, J = 7.6 Hz), 1.95 - 1.90 (m,

4H), 1.54 (p, 2H,  $J = 7.4$  Hz), 1.43 (s, 2H,  $J = 7.2$  Hz), 1.21 - 1.19 (m, 20H), 0.89 - 0.78 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  173.1, 129.8, 129.6, 41.0, 36.6, 31.7, 29.60, 29.56, 29.4, 29.2 (4), 29.0, 27.04, 27.01, 25.7, 22.7, 22.5, 13.9, 11.2; IR (film)  $\nu_{\text{max}}$  3298, 2924, 2854, 1648, 1552, 1464, 1378, 1253, 1153, 757  $\text{cm}^{-1}$ ; ESI ( $\text{M} + \text{H}^+$ ) 324.

***i*-PrNH derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma: white solid: mp 25 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.46 - 5.45 (br, 1H), 5.33 - 5.24 (m, 2H), 4.07 - 3.96 (m, 1H), 2.07 (t, 2H,  $J = 7.6$  Hz), 1.97 - 1.93 (m, 4H), 1.56 (p, 2H,  $J = 7.1$  Hz), 1.24 - 1.15 (m, 20H), 1.08 (d, 6H,  $J = 6.6$  Hz), 0.83 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  172.2, 129.9, 129.7, 41.0, 36.9, 31.8, 29.7, 29.6, 29.4, 29.23, 29.19, 29.1, 27.11, 27.08, 25.7, 22.7, 22.6, 14.0; IR (film)  $\nu_{\text{max}}$  3291, 2925, 2854, 1645, 1558, 1540, 1457, 1174, 1130, 722  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  324.3259 ( $\text{C}_{21}\text{H}_{41}\text{NO} + \text{H}^+$  requires 324.3266).

**$\text{CH}_2=\text{CHCH}_2\text{NH}$  derivative as illustrated in Figure 3** prepared as described in the literature (Synth. Comm. 1996, 26, 2341-2348).

25 ***i*-PrNMe derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vide supra* using *i*-Propyl-N-methylamine; all starting reagents were purchased from Aldrich, Acros, or Sigma.

30

**cyclopropylamine derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vide supra* using the cyclopropyl amine; all starting reagents were purchased from

Aldrich, Acros, or Sigma.

**Ph(CH<sub>2</sub>)<sub>3</sub>NH derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives  
5 varying about carboxamide vida supra using the commercially available Ph(CH<sub>2</sub>)<sub>3</sub>NH amine; all starting reagents were purchased from Aldrich, Acros, or Sigma.

**BuNH derivative as illustrated in Figure 3** Synthesized exactly  
10 as found in the general procedure for derivatives varying about carboxamide vida supra; all starting reagents were purchased from Aldrich, Acros, or Sigma: white solid: mp 30-31 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.13 (br, 1H), 5.29 - 5.20 (m, 2H), 3.14 (q, 2H, J = 5.9 Hz), 2.07 (t, 2H, J = 7.6 Hz), 1.92 - 1.89 (m, 4H),  
15 1.53 (p, 2H, J = 6.8 Hz), 1.43 - 1.18 (m, 24H), 0.85 - 0.77 (m, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 173.1, 129.8, 129.5, 39.0, 36.6, 31.7, 31.6, 29.59, 29.55, 29.4, 29.2, 29.0, 27.03, 27.00, 25.7, 22.6, 19.9, 13.9, 13.5; IR (film) ν<sub>max</sub> 3301, 3084, 3001, 2954, 2918, 2849, 1639, 1559, 1466, 1231, 720 cm<sup>-1</sup>; FABHRMS (NBA-NaI)  
20 m/z 338.3428 (C<sub>22</sub>H<sub>43</sub>NO + H<sup>+</sup> requires 338.3423).

**pyrrole derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives  
varying about carboxamide vida supra; all starting reagents were  
25 purchased from Aldrich, Acros, or Sigma: Pale yellow oil was purified by chromatography (33% EtOAc/hexanes) to afford desired product in quantitative yield. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 5.29 - 5.21 (m, 2H), 3.39 (t, 2H, J = 6.7 Hz), 3.31 (t, 2H, J = 6.9 Hz), 2.22 (t, 2H, J = 7.6 Hz), 1.94 (m, 4H), 1.88 (p, 2H, J =  
30 6.7 Hz), 1.78 (p, 2H, J = 6.9 Hz), 1.52 (p, 2H, J = 7.2 Hz), 1.25 - 1.20 (m, 20H), 0.81 (t, 3H, J = 6.8 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz) δ 173.9, 130.83, 130.79, 47.9, 46.8, 35.5, 33.1, 30.94, 30.90, 30.7, 30.54, 30.46, 30.3, 28.2, 27.0, 26.1, 25.4,

23.8, 14.7; IR (NaCl, film)  $\nu_{\max}$  2924, 2853, 1651, 1428, 1342, 1226, 1194, 723  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $M+\text{Cs}^+$ ) 468.2225 (requires  $m/z$  468.2242).

- 5 **PhNH derivative as illustrated in Figure 3** prepared as described in the literature (Synth. Comm. 1995, 25, 959-968).

**NHOH derivative as illustrated in Figure 3:** prepared as previously described (JACS, 1996, 118, 5938-5945).

10

- MeONMe derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma:: Pale yellow oil was
- 15 purified by chromatography (33% EtOAc/hexanes) to afford desired product in quantitative yield.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  5.34 - 5.26 (m, 2H), 3.66 (s, 3H), 3.12 (s, 3H), 2.39 (t, 3H,  $J = 7.0$  Hz), 1.99 (m, 4H), 1.56 (p, 2H,  $J = 6.8$  Hz), 1.30 - 1.26 (m, 20H), 0.86 (t, 3H,  $J = 6.1$  Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  176.2,
- 20 130.9, 130.8, 61.8, 33.1, 32.7, 30.92, 30.88, 30.77, 30.54, 30.49, 30.45, 30.3, 28.2, 25.8, 23.8, 14.6; IR (NaCl, film)  $\nu_{\max}$  2923, 2853, 1673, 1463, 1413, 1383, 1177, 1116, 998, 722  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $M+\text{H}^+$ ) 326.3072 (requires  $m/z$  326.3059).

- 25 **NH<sub>2</sub>NH derivative as illustrated in Figure 3:** prepared as previously described (JACS, 1996, 118, 5938-5945).

- MeO (methyl oleate) derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for
- 30 derivatives varying about carboxamide *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma: The crude mixture was purified by chromatography ( $\text{SiO}_2$ , 5 x 15 cm, 1% EtOAc/hexanes) to afford quantitative yield of a clear oil.  $^1\text{H}$

NMR spectra agrees with spectra in Sadtler Handbook of NMR data. Additional data:  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  176.0, 130.9, 130.8, 52.0, 34.8, 33.1, 30.9, 30.8, 30.6, 30.5, 30.4, 30.3, 30.20, 30.17, 28.14, 28.11, 26.1, 23.8, 14.5; IR (NaCl, film)  $\nu_{\text{max}}$  2924, 2854, 1743, 1653, 1558, 1540, 1506, 1457, 1260, 1093, 1018, 801  $\text{cm}^{-1}$ .

**EtO (ethyl oleate) derivative as illustrated in Figure 3**  
Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vida supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma: clear oil.  $^1\text{H}$  NMR spectra agrees with spectra in Sadtler Handbook of NMR data. Additional data:  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  173.8, 129.9, 129.7, 60.1, 34.3, 31.9, 29.7, 29.6, 29.5, 29.3, 29.12, 29.08, 29.05, 27.2, 27.1, 24.9, 22.6, 14.2, 14.1; IR (NaCl, film)  $\nu_{\text{max}}$  2925, 2854, 1739, 1465, 1373, 1244, 1180, 1036, 723  $\text{cm}^{-1}$ .

**$\text{Me}_2\text{CHCH}_2\text{O}$  derivative as illustrated in Figure 3** Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vida supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma: pale yellow oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.35 - 5.27 (m, 2H), 3.82 (d, 2H,  $J = 3.4$  Hz), 2.27 (t, 2H,  $J = 7.5$  Hz), 1.98 - 1.95 (m, 4H), 1.89 (septet, 1H,  $J = 6.7$  Hz), 1.59 (quintet, 2H,  $J = 6.9$  Hz), 1.27 - 1.23 (m, 20H), 0.90 (d, 6H,  $J = 3.4$  Hz), 0.85 (t, 3H,  $J = 6.6$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  173.97, 129.92, 129.72, 70.36, 34.36, 31.89, 29.75, 29.67, 29.51, 29.31 (2), 29.15, 29.13, 29.09, 27.70, 27.19, 27.14, 25.02, 22.67, 19.07 (2), 14.10; IR (NaCl, film)  $\nu_{\text{max}}$  2925.2, 2853.9, 1739.7, 1466.2, 1378.6, 1172.6, 1012.6; FABMS  $m/z$  ( $\text{M}+\text{H}^+$ ) 339.

**H(oley)l aldehyde as illustrated in Figure 3:** prepared as described in the literature (JOC 1978, 43,2480-2482).

CF<sub>3</sub> as illustrated in Figure 3: prepared as previously described (JACS, 1996, 118, 5938-5945).

ClCH<sub>2</sub> as illustrated in Figure 3: prepared as previously  
5 described (JACS, 1996, 118, 5938-5945).

N<sub>3</sub>CH<sub>2</sub> as illustrated in Figure 3: prepared as previously described (JACS, 1996, 118, 5938-5945).

10 Oleyl alcohol as illustrated in Figure 3: prepared as described in the literature (JOC 1978, 43, 2480-2482).

Oleyl acetate: purchased from Sigma

15 Oleyl amine: purchased from Pfaltz and Bauer

Oleyl aldehyde dimethyl acetal as illustrated in Figure 3: prepared as described in the literature (J Med Chem, 1989, 32, 1319-1322).

20

CoA-SCO derivative as illustrated in Figure 3: purchased from Sigma

25 HOCH<sub>2</sub>CH<sub>2</sub>NH derivative as illustrated in Figure 4: Prepared as previously described. (JACS, 1996, 118, 580-590).

(HOCH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>NH derivative as illustrated in Figure 4: Purchased from Pfaltz and Bauer.

30 HOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH derivative as illustrated in Figure 4 Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma: white solid: mp 55-56 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.87 (t, 1H, J = 5.8 Hz), 5.28 -



5.19 (m, 2H), 4.25 (br, 1H), 3.52 (s, 2H), 3.27 (q, 2H,  $J = 6.2$  Hz), 2.09 (t, 2H,  $J = 7.6$  Hz), 1.91 - 1.88 (m, 4H), 1.58 (p, 4H,  $J = 6.0$  Hz), 1.51 (p, 2H,  $J = 7.1$  Hz), 1.20 - 1.17 (m, 20H), 0.78 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  174.6, 129.8, 129.4, 58.9, 36.4, 36.0, 31.9, 31.7, 29.5, 29.3, 29.1, 29.0, 27.00, 26.96, 25.7, 22.5, 13.9; IR (film)  $\nu_{\text{max}}$  3290, 2917, 2848, 1634, 1550, 1462, 1412, 1216, 1052, 762  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  340.3226 ( $\text{C}_{21}\text{H}_{41}\text{NO}_2 + \text{H}^+$  requires 340.3216).

10  $\text{HOCH}_2\text{CH}(\text{OAc})\text{CH}_2\text{O}$  derivative as illustrated in Figure 4:

Purchased from Sigma

$\text{HOCH}_2\text{CH}_2\text{NH}$  derivative as illustrated in Figure 4: Purchased from Sigma

15  $(\text{HOCH}_2\text{CH}_2)_2\text{NH}$  derivative as illustrated in Figure 4 Synthesized exactly as found in the general procedure for derivatives varying about carboxamide *vide supra*; all starting reagents were purchased from Aldrich, Acros, or Sigma: orange oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.68 (br, 1H), 7.50 (br, 1H), 5.38 - 5.29 (m, 8H), 3.78 (t, 2H,  $J = 5.0$  Hz), 3.73 (t, 2H,  $J = 5.3$  Hz), 3.50 (t, 2H,  $J = 5.0$  Hz), 3.45 (t, 2H,  $J = 5.0$  Hz), 2.81 - 2.77 (m, 6H), 2.36 (t, 2H,  $J = 7.5$  Hz), 2.09 (q, 2H,  $J = 6.0$  Hz), 2.02 (q, 2H,  $J = 7.0$  Hz), 1.67 (p, 2H,  $J = 7.5$  Hz), 1.34 - 1.22 (m, 6H), 0.85 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  175.3, 130.5, 129.2, 128.7, 128.5, 128.1 (2), 127.8, 127.4, 61.3, 60.7, 52.2, 50.5, 32.8, 31.4, 29.3, 27.2, 26.6, 25.6 (3), 25.0, 22.5, 14.0; IR (film)  $\nu_{\text{max}}$  3375, 3011, 2956, 2926, 2857, 1727, 1621, 1455, 1270, 1072  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  524.2152 ( $\text{C}_{24}\text{H}_{41}\text{NO}_3 + \text{Cs}^+$  requires 524.2141).

30

$\text{CH}_2\text{OCH}_3$  derivative as illustrated in Figure 5 and scheme shown in Figure 19 (left side): A solution of 18-TBDPS-acid (prepared as described in JACS, 1996, 118, 580-590) (390.1 mg, 0.58 mmol, 1

eq) in THF (2 mL) under N<sub>2</sub> at 25 °C was treated with TBAF (1M in THF, 1.2 mL, 1.2 mmol, 2 eq) for 2 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 2 x 8 cm, 20 - 100% EtOAc/hexanes, gradient elution) to afford **18-OH-acid** (149.1 mg, 86%) as a white solid: 25 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.47 (br, 1H), 5.35 - 5.26 (m, 2H), 3.60 (t, 2H, J = 6.7 Hz), 2.29 (t, 2H, J = 7.5 Hz), 1.98 - 1.95 (m, 4H), 1.60 - 1.49 (m, 4H), 1.27 (s, 18H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 179.2, 129.9, 129.8, 62.8, 34.1, 32.5, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0 (2), 27.11, 27.07, 25.7, 24.7; IR (film) ν<sub>max</sub> 3355, 2926, 2853, 1710, 1462, 1409, 1246, 1054, 722 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 321.2415 (C<sub>18</sub>H<sub>34</sub>O<sub>3</sub> + Na<sup>+</sup> requires 321.2406).

15 A solution of **18-OH-acid** (67.7 mg, 0.23 mmol, 1 eq) in anhydrous THF (2.2 mL) under N<sub>2</sub> at 0 °C was treated with NaH (60%, 28.1 mg, 0.70 mmol, 3 eq) for 15 min. MeI (72 μL, 1.16 mmol, 5 eq) was added and the reaction was warmed to 25 °C and allowed to stir for 5 h. A second portion of MeI (140 μL, 2.25 mmol, 10 eq) was added and the reaction was stirred for an additional 14 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 2 x 8 cm, 20 - 100% EtOAc/hexanes, gradient elution) to afford **18-methoxy-acid** (41.0 mg, 58%) as a clear oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.35 - 5.27 (m, 2H), 3.35 (t, 2H, J = 6.7 Hz), 3.31 (s, 3H), 2.31 (t, 2H, J = 7.5 Hz), 1.99 - 1.96 (m, 4H), 1.64 - 1.50 (m, 4H), 1.28 - 1.27 (m, 18H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 179.7, 129.9, 129.7, 73.0, 58.4, 34.0, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 29.0, 27.2, 27.1, 26.1, 24.7; IR (film) ν<sub>max</sub> 2926, 2854, 1710, 1458, 1119 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 335.2568 (C<sub>19</sub>H<sub>36</sub>O<sub>3</sub> + Na<sup>+</sup> requires 335.2562).

A solution of **18-methoxy-acid** (37.7 mg, 0.12 mmol, 1 eq) in

anhydrous  $\text{CH}_2\text{Cl}_2$  (0.6 mL) under  $\text{N}_2$  at  $0^\circ\text{C}$  was treated with oxalyl chloride (2M in  $\text{CH}_2\text{Cl}_2$ , 0.18 mL, 0.36 mmol, 3 eq). The reaction mixture was warmed to  $25^\circ\text{C}$  and stirred for 3 h before the solvent was removed in vacuo. The residue was cooled to  $0^\circ\text{C}$  and treated with excess concentrated  $\text{NH}_4\text{OH}$  (1 mL). Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 2 x 5 cm, 50 - 100% EtOAc/hexanes, gradient elution) to afford  $\text{CH}_2\text{OCH}_3$  (34.6 mg, 92%) as a white solid: mp  $59-60^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.71 (br, 1H), 5.52 (br, 1H), 5.35 - 5.27 (m, 2H), 3.33 (t, 2H,  $J = 6.7$  Hz), 3.29 (s, 3H), 2.18 (t, 2H,  $J = 7.6$  Hz), 1.98 - 1.96 (m, 4H), 1.61 - 1.49 (m, 4H), 1.27 - 1.26 (m, 18H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.8, 129.9, 129.8, 72.9, 58.5, 35.9, 29.7, 29.64, 29.60, 29.4, 29.20, 29.18, 29.07, 27.14, 27.12, 26.1, 25.5; IR (film)  $\nu_{\text{max}}$  3356, 3191, 2922, 2851, 2358, 1660, 1633, 1469, 1410, 1120  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  334.2730 ( $\text{C}_{19}\text{H}_{37}\text{O}_2\text{N} + \text{Na}^+$  requires 334.2722).

$\text{CH}_2\text{OH}$  derivative as illustrated in Figure 5 is commercially available from Aldrich.

$\text{CONH}_2$  derivative as illustrated in Figure 5 and scheme shown in Figure 19 (left side): A solution of  $\text{CO}_2\text{H}$  (73.8 mg, 0.24 mmol, 1 eq) in anhydrous  $\text{CH}_2\text{Cl}_2$  (1.2 mL) under  $\text{N}_2$  at  $0^\circ\text{C}$  was treated with oxalyl chloride (2M in  $\text{CH}_2\text{Cl}_2$ , 0.36 mL, 0.72 mmol, 3 eq). The reaction mixture was warmed to  $25^\circ\text{C}$  and stirred for 3 h in the dark before the solvent was removed in vacuo. The residue was cooled to  $0^\circ\text{C}$  and treated with excess concentrated  $\text{NH}_4\text{OH}$  (3 mL). Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL) and  $\text{CHCl}_3$  (3 x 30 mL). The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 2 x 15 cm, 0 - 10% MeOH/EtOAc, gradient elution) to afford  $\text{CONH}_2$  (27.8 mg, 38%) as a white

solid: mp 128-129 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.47 (br, 4H), 5.35 - 5.28 (m, 2H), 2.20 (t, 4H, J = 7.6 Hz), 1.98 (q, 4H, J = 5.6 Hz), 1.61 (p, 4H, J = 7.3 Hz), 1.29 (s, 12H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 175.6 (2), 129.9 (2), 35.9 (2), 29.6 (2), 29.2 (4), 29.0 (2), 27.1 (2), 25.5 (2); IR (film)  $\nu_{\max}$  3385, 3186, 2921, 2848, 1647, 1419 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 311.2691 (C<sub>18</sub>H<sub>34</sub>N<sub>2</sub>O<sub>2</sub> + H<sup>+</sup> requires 311.2699).

**CO<sub>2</sub>H derivative as illustrated in Figure 5:** A solution of 18-hydroxy-amide (prepared as described in JACS, 1996, 118, 580-590) (101.2 mg, 0.34 mmol, 1 eq) in anhydrous DMF (3.4 mL) under N<sub>2</sub> was treated with PDC (644.0 mg, 1.71 mmol, 5 eq) at 25 °C for 5 h. Water (30 mL) was added and the mixture was extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 2.5 x 15 cm, 5 - 10% EtOAc/hexanes, gradient elution) to afford CO<sub>2</sub>H (85.0 mg, 80%) as a white solid: mp 74-76 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.71 (br, 1H), 5.74 (br, 1H), 5.34 - 5.27 (m, 2H), 2.29 (t, 2H, J = 7.5 Hz), 2.19 (t, 2H, J = 7.6 Hz), 1.97 (m, 4H), 1.59 (m, 4H), 1.27 (s, 16H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 178.6, 177.5, 129.9, 129.8, 35.9, 34.2, 29.6, 29.5, 29.2, 29.10, 29.08, 29.02 (2), 28.9, 27.1, 27.0, 25.5, 24.8; IR (film)  $\nu_{\max}$  3389, 3191, 3008, 2917, 2848, 1704, 1645, 1466, 1417 cm<sup>-1</sup>; FABHRMS (NBA-CsI) m/z 444.1524 (C<sub>18</sub>H<sub>33</sub>NO<sub>3</sub> + Cs<sup>+</sup> requires 444.1515).

**OH derivative as illustrated in Figure 6:** Oleic glycine ethyl ester (OEt) (725.5 mg, 1.97 mmol, 1 equiv) was dissolved in 6 mL of THF/MeOH/H<sub>2</sub>O (3:1:1). The solution was treated with LiOH·H<sub>2</sub>O (236.6 mg, 5.63 mmol, 2.9 equiv) and stirred at 25 °C for 15 minutes. The solution was diluted with 1N HCl (30 mL) and extracted with EtOAc (3 x 30 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo to afford (611.2 mg, 91%) of OH as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)

$\delta$  7.04 (br, 1H), 6.26 (t, 1H,  $J = 4.8$  Hz), 5.36 - 5.27 (m, 2H), 4.04 (d, 2H,  $J = 5.2$  Hz), 2.24 (t, 3H,  $J = 7.7$  Hz), 1.97 (m, 4H), 1.61 (p, 2H,  $J = 7.2$  Hz), 1.27 - 1.24 (m, 20H), 0.85 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  174.6, 172.7, 130.0, 129.7, 41.5, 36.3, 31.9, 29.74, 29.68, 29.5, 29.3 (2), 29.19, 29.15, 29.1, 27.20, 27.15, 25.5, 22.7, 14.1; IR (NaCl, film)  $\nu_{\text{max}}$  3429, 3304, 2918, 2849, 1699, 1645, 1552, 1469, 1410, 1239, 718, 677  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $\text{M}+\text{H}^+$ ) 340.2861 (requires  $m/z$  340.2852); mp 90-91  $^{\circ}\text{C}$ .

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**NH<sub>2</sub> derivative as illustrated in Figure 6** Oleic glycine (OH) (325.6 mg, .96 mmol, 1 equiv), dry  $\text{CH}_2\text{Cl}_2$  (16 mL), and concentrated ammonium hydroxide (225  $\mu\text{L}$ ) were combined under a  $\text{N}_2$  atmosphere and cooled to 0  $^{\circ}\text{C}$ . EDCI (573.1 mg, 1.93 mmol, 1 equiv) and DMAP (24.4 mg, 0.20 mmol, 0.2 equiv) were added and the reaction was stirred at 25  $^{\circ}\text{C}$  for 2.5 h. The solvent was removed in vacuo and the crude product was purified by chromatography ( $\text{SiO}_2$ , 3 x 18 cm, 0-10% methanol/EtOAc, gradient elution) to afford 231.1 mg (71%) of **NH<sub>2</sub>** as a white solid.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  8.03 (br, 1H), 5.28 - 5.20 (m, 2H), 3.72 (d, 2H,  $J = 1.4$  Hz), 2.16 (t, 2H,  $J = 7.6$  Hz), 1.92 (m, 4H), 1.52 (p, 2H,  $J = 7.2$  Hz), 1.23 - 1.19 (m, 20H), 0.80 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  173.8, 171.0, 130.0, 129.7, 42.8, 36.4, 31.9, 29.8, 29.7, 29.5, 29.3, 29.2, 29.1, 27.21, 27.16, 25.6, 22.7, 14.1; IR (NaCl, film)  $\nu_{\text{max}}$  3385, 3307, 3195, 2918, 2849, 1661, 1643, 1549, 1468, 1415  $\text{cm}^{-1}$ ; HRFABMS  $m/z$  ( $\text{M}+\text{H}^+$ ) 339.3020 (requires  $m/z$  339.3012); mp 124-125  $^{\circ}\text{C}$ .

**OEt derivative as illustrated in Figure 6:** Oleic acid (100  $\mu\text{L}$ , .32 mmol, 1 equiv), glycine ethyl ester hydrochloride (45.9 mg, .33 mmol, 1 equiv), dry triethylamine (66  $\mu\text{L}$ , .47 mmol, 1.5 equiv) and dry methylene chloride (5 mL) were combined under a  $\text{N}_2$  atmosphere and cooled to 0  $^{\circ}\text{C}$ . EDCI (107.0 mg, 0.36 mmol, 1.1 equiv) and DMAP (8.1 mg, 0.07 mmol, 0.2 equiv) were added and

30

the reaction was allowed to stir at 25 °C for 4 h. The solvent was removed in vacuo and the crude product was chromatographed (SiO<sub>2</sub>, 2 x 15 cm, 20% - 33% EtOAc/hexanes, gradient elution) to afford OEt as a white solid (109.5 mg, 94%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.95 (br, 1H), 5.36 - 5.27 (m, 2H), 4.19 (quartet, 2H, *J* = 7.1 Hz), 4.00 (d, 2H, *J* = 5.1 Hz), 2.21 (t, 2H, *J* = 7.6 Hz), 1.99 (m, 4H), 1.62 (p, 2H, *J* = 7.2 Hz), 1.28 - 1.24 (m, 23H), 0.85 (t, 3H, *J* = 6.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 173.2, 170.1, 130.0, 129.7, 61.5, 41.3, 36.4, 31.9, 29.73, 29.67, 29.5, 29.3, 29.2, 29.1, 27.2, 27.1, 25.5, 22.7, 14.1; IR (NaCl, film)  $\nu_{\max}$  3311, 2918, 2849, 1739, 1647, 1552, 1465, 1413, 1375, 1213, 1032, 700 cm<sup>-1</sup>; HRFABMS *m/z* (M+H<sup>+</sup>) 368.3175 (requires *m/z* 368.3165); mp 32-33 °C.

15 OH/ R<sub>1</sub> = Me derivative as illustrated in Figure 6: purchased from Sigma

OEt/ R<sub>1</sub> = Me derivative as illustrated in Figure 6: (both isomers): To a solution of oleoyl sarcosine (OH) (200.7 mg, 0.57 mmol, 1 eq) in anhydrous THF (6 mL) was added DCC (158.9 mg, 0.77 mmol, 1.4 eq) and absolute EtOH (50 μL, 0.85 mmol, 1.5 eq). The reaction mixture was stirred at 25 °C for 22 h. The white precipitate was filtered off and water (30 mL) was added to the eluent. The aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 3 x 15 cm, 10 - 50% EtOAc/hexanes, gradient elution) to afford clear oil OEt (150.8 mg, 70%) as a mixture of isomers; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.30 - 5.27 (m, 2H), 4.19 - 4.09 (m, 2H), 4.05 + 3.97 (s, 2H), 3.01 + 2.91 (s, 3H), [2.31 (t, *J* = 7.5 Hz) + 2.16 (t, *J* = 7.4 Hz)] [2H], 1.96 - 1.94 (m, 4H), 1.59 (p, 2H, *J* = 6.6 Hz), 1.26 - 1.20 (m, 23 H), 0.82 (t, 3H, *J* = 6.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) major isomer (minor isomer) δ 173.7 (173.3), 169.3 (169.0), 129.8, 129.7, (127.9, 127.8), (61.4), 60.9,



(51.6), 49.3, 36.4, (34.7), 33.0, (32.8, 32.5), 31.8, (31.4),  
29.64, 29.60, 29.4, 29.23, 29.19, 29.0, 27.1, (24.9), 24.8,  
22.6, 14.03, 13.99; IR (film)  $\nu_{\max}$  2924, 2853, 1750, 1655, 1465,  
1400, 1373, 1197, 1113, 1035  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$   
5 404.3128 ( $\text{C}_{23}\text{H}_{43}\text{O}_3\text{N} + \text{Na}^+$  requires 404.3141).

**2(9-octadecynamide) derivative as illustrated in Figure 7**

Synthesized exactly as found in the general procedure prepared  
in a manner analogous to 6; all starting reagents were purchased  
10 from Aldrich, Acros, or Sigma: mp 91-92 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400  
MHz)  $\delta$  6.14 (br, 1H), 5.62 (br, 1H), 2.15 (t, 2H,  $J = 7.6$  Hz),  
2.07 (t, 4H,  $J = 7.0$  Hz), 1.57 (p, 2H,  $J = 7.1$  Hz), 1.41 (p, 4H,  
 $J = 7.4$  Hz), 1.35 - 1.21 (m, 16H), 0.82 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$   
NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  176.0, 80.2, 80.0, 35.9, 31.8, 29.1, 29.04  
15 (3), 28.98, 28.8 (2), 28.6, 25.4, 22.6, 18.7, 18.6, 14.0; IR  
(film)  $\nu_{\max}$  3409, 2928, 2850, 1647, 1471, 1419, 712  $\text{cm}^{-1}$ ; FABHRMS  
(NBA-CsI)  $m/z$  412.1636 ( $\text{C}_{18}\text{H}_{33}\text{NO} + \text{Cs}^+$  requires 412.1616).

**3 derivative as illustrated in Figure 7:** Prepared as previously  
20 described. (JACS, 1996, 118, 580-590).

**4 derivative as illustrated in Figure 7:** Prepared as described  
in the literature (JACS, 1959, 81, 4256).

25 **5 derivative as illustrated in Figure 7** Synthesized exactly as  
found in the general procedure prepared in a manner analogous to  
6; all starting reagents were purchased from Aldrich, Acros, or  
Sigma: mp 47-48 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.13 - 7.07 (m,  
4H), 5.53 (br, 1H), 5.41 (br, 1H), 2.57 (td, 4H,  $J = 7.9, 1.5$   
30 Hz), 2.20 (t, 2H,  $J = 7.6$  Hz), 1.62 (p, 2H,  $J = 7.1$  Hz), 1.55  
(p, 4H,  $J = 7.8$  Hz), 1.34 - 1.26 (m, 16H), 0.86 (t, 3H,  $J = 6.8$   
Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.6, 140.5, 140.4, 129.1,  
129.0, 125.70, 125.66, 35.9, 32.7, 32.6, 31.9, 31.3, 31.2, 29.8,

29.6, 29.5, 29.3 (2), 29.2, 25.5, 22.7, 14.1; IR (film)  $\nu_{\max}$  3392, 3194, 2924, 2853, 1647, 1464, 1415, 746  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  354.2776 ( $\text{C}_{22}\text{H}_{37}\text{NO}$  requires 354.2773).

5 **6 derivative as illustrated in Figure 7 and scheme shown on Figure 20:** A solution of 1-bromopentane (1.0 mL, 8.1 mmol, 1 eq) and  $\text{Ph}_3\text{P}$  (2.32 g, 8.8 mmol, 1.1 eq) in anhydrous  $\text{CH}_3\text{CN}$  (6 mL) under  $\text{N}_2$  was refluxed for 20 h. The reaction mixture was concentrated in vacuo and the crude product was chromatographed  
10 ( $\text{SiO}_2$ , 3 x 20 cm, 10% EtOAc/hexanes - 50% iPrOH/EtOAc, gradient elution) to afford **phosphonium salt** (3.27 g, 98%) as a white foam;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.84 - 7.73 (m, 9H), 7.69 - 7.65 (m, 6H), 3.78 - 3.71 (m, 2H), 1.58 - 1.57 (m, 4H), 1.25 (s, 2H,  $J = 7.2$  Hz), 0.78 (t, 3H,  $J = 7.3$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$   
15 134.9 (3, d,  $J = 2$  Hz), 133.6 (6, d,  $J = 10$  Hz), 130.4 (6, d,  $J = 12$  Hz), 118.3 (3, d,  $J = 86$  Hz), 32.3 (d,  $J = 15$  Hz), 22.9, 22.3, 22.2 (d,  $J = 3$  Hz), 13.6; IR (film)  $\nu_{\max}$  3405, 2921, 2864, 1438, 1112, 996, 749, 723, 690  $\text{cm}^{-1}$ ; ESI ( $\text{M} - \text{Br}^+$ ) 333.

A solution of **phosphonium salt** (3.2 g, 7.74 mmol, 1.1 eq)  
20 in anhydrous THF (60 mL) under  $\text{N}_2$  at  $-78^\circ\text{C}$  was treated with  $n\text{-BuLi}$  (2.0M, 7.0 mL, 14.0 mmol, 2.0 eq). The reaction mixture was warmed to  $25^\circ\text{C}$  and allowed to stir for 10 min. The reaction was recooled to  $-78^\circ\text{C}$  and treated with a solution of 2-cyanobenzaldehyde (0.9119 g, 6.95 mmol, 1 eq) in anhydrous THF  
25 (15 mL). The reaction mixture was then warmed to  $25^\circ\text{C}$  and stirred for 1 h before an aqueous solution of saturated  $\text{NH}_4\text{Cl}$  (100 mL) was added. The aqueous layer was extracted with EtOAc (3 x 100 mL) and the organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was  
30 chromatographed ( $\text{SiO}_2$ , 6 x 20 cm, 0-5% EtOAc/hexanes, gradient elution) to afford **CN-alkene** (0.9796 g, 76%) as a clear oil (mixture of E/Z isomers);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.64 - 7.22 (m, 4H), [6.72 (d,  $J = 15.7$  Hz) + 6.59 (d,  $J = 11.6$  Hz)] [1H], [6.41 (dt,  $J = 15.7, 7.0$  Hz) + 5.90 (dt,  $J = 11.6, 7.5$  Hz)] [1H],

[2.27 (qd,  $J = 7.6, 1.4$  Hz) + 2.21 (qd,  $J = 7.3, 1.7$  Hz)] [2H],  
1.50 - 1.20 (m, 4H), [0.91 (t,  $J = 7.2$  Hz) + 0.84 (t,  $J = 7.2$   
Hz)] [3H];  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  141.3, 141.2, 137.4, 136.8,  
132.8, 132.7, 132.6, 132.2, 129.4, 126.8, 125.8, 125.3, 125.0,  
5 118.1, 118.0, 112.2, 110.4, 32.8, 31.6, 31.1, 28.4, 22.2, 13.9,  
13.8; IR (film)  $\nu_{\text{max}}$  2957, 2928, 2858, 2224, 1647, 1596, 1478,  
1466, 1448, 966, 759  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  186.1285  
( $\text{C}_{13}\text{H}_{15}\text{N} + \text{H}^+$  requires 186.1283).

A solution of **CN-alkene** (0.9394 g, 5.1 mmol, 1 eq) in  
10 anhydrous toluene (10 mL) under  $\text{N}_2$  at 0 °C was treated with DIBAL  
(1M, 5.6 mL, 5.6 mmol, 1.1 eq). The reaction mixture was  
stirred for 5 min before an aqueous saturated solution of  $\text{NH}_4\text{Cl}$   
(60 mL) was added. The mixture was stirred at 25 °C for 15 min  
before the aqueous layer was extracted with EtOAc (3 x 60 mL).  
15 The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and  
concentrated in vacuo. The crude product was chromatographed  
( $\text{SiO}_2$ , 4 x 20 cm, 0-4% EtOAc/hexanes, gradient elution) to afford  
**CHO-alkene** (0.86 g, 91%) as a clear oil (mixture of E/Z  
isomers);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  [10.26 + 10.22] [s, 1H],  
20 [7.87 (dd,  $J = 7.8, 1.4$  Hz) + 7.77 (dt,  $J = 7.6, 0.9$  Hz)] [1H],  
7.54 - 7.23 (m, 3H), [7.15 (dt,  $J = 15.6, 1.5$  Hz) + 6.77 (d,  $J =$   
11.5 Hz)] [1H], [6.13 (dt,  $J = 15.6, 6.9$  Hz) + 5.90 (dt,  $J =$   
11.5, 7.5 Hz)] [1H], [2.26 (qd,  $J = 7.5, 1.5$  Hz) + 2.03 (qd,  $J =$   
7.3, 1.6 Hz)] [2H], 1.50 - 1.18 (m, 4H), [0.91 (t,  $J = 7.3$  Hz) +  
25 0.79 (t,  $J = 7.3$  Hz)] [3H];  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  192.4,  
141.0, 137.5, 136.2, 133.6, 133.5, 132.5, 130.5, 130.4, 128.4,  
127.4, 127.1, 126.9, 125.6, 125.1, 33.0, 31.4, 31.2, 28.1,  
22.21, 22.16, 13.9, 13.8; IR (film)  $\nu_{\text{max}}$  2957, 2928, 2871, 1695,  
1597, 1566, 1480, 1466, 1451, 1389, 1289, 1196, 968, 762  $\text{cm}^{-1}$ ;  
30 FABHRMS (NBA-NaI)  $m/z$  189.1280 ( $\text{C}_{13}\text{H}_{16}\text{O} + \text{H}^+$  requires 189.1279).

A solution of 5-bromo-pentanol (0.9889 g, 5.9 mmol, 1 eq)  
in anhydrous  $\text{CH}_2\text{Cl}_2$  (20 mL) under  $\text{N}_2$  at 25 °C was treated with  
anhydrous  $\text{Et}_3\text{N}$  (1.0 mL, 7.2 mmol, 1.2 eq), TBDPSCl (1.7 mL, 6.5  
mmol, 1.1 eq) and DMAP (0.23 g, 1.9 mmol, 0.3 eq). The reaction

mixture was stirred for 2 h before an aqueous solution of saturated  $\text{NH}_4\text{Cl}$  (100 mL) was added. The aqueous layer was extracted with EtOAc (3 x 100 mL) and the organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude  
5 product was chromatographed ( $\text{SiO}_2$ , 5 x 20 cm, 0-20% EtOAc/hexanes, gradient elution) to afford **TBDPSOH** (2.35 g, 98%) as a clear oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.66 - 7.60 (m, 4H), 7.44 - 7.33 (m, 6H), 3.64 (t, 2H,  $J$  = 6.1 Hz), 3.37 (t, 2H,  $J$  = 6.9 Hz), 1.82 (p, 2H,  $J$  = 7.3 Hz), 1.59 - 1.45 (m, 4H), 1.03 (s, 9H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  135.6, 129.6, 127.6, 127.5, 63.5, 33.9, 32.5, 31.6, 26.8, 24.5, 19.2; IR (film)  $\nu_{\text{max}}$  3069, 2930, 2856, 2359, 1470, 1427, 1389, 1106, 822, 738, 700  $\text{cm}^{-1}$ ; FABHRMS (NBA- $\text{CsI}$ )  $m/z$  537.0235 ( $\text{C}_{21}\text{H}_{29}\text{OSiBr} + \text{Cs}^+$  requires 537.0225).

A solution of **TBDPSOH** (2.35 g, 5.8 mmol, 1 eq) and  $\text{Ph}_3\text{P}$  (1.67  
15 g, 6.4 mmol, 1.1 eq) in anhydrous  $\text{CH}_3\text{CN}$  (6 mL) under  $\text{N}_2$  was refluxed for 22 h. The reaction mixture was concentrated in vacuo and the crude product was chromatographed ( $\text{SiO}_2$ , 5 x 20 cm, 20% EtOAc/hexanes - 50%  $i\text{PrOH}$ /EtOAc, gradient elution) to afford **TBDPSphosphonium salt** (2.82 g, 73%) as a white foam;  $^1\text{H}$  NMR  
20 ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.84 - 7.72 (m, 9H), 7.67 - 7.62 (m, 6H), 7.58 - 7.55 (m, 4H), 7.38 - 7.29 (m, 6H), 3.84 (m, 2H), 3.57 (t, 2H,  $J$  = 6.3 Hz), 1.75 - 1.51 (m, 6H), 0.95 (s, 9H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  135.4 (4), 134.9 (3, d,  $J$  = 3 Hz), 133.8 (2), 133.7 (6, d,  $J$  = 10 Hz), 130.4 (6, d,  $J$  = 13 Hz), 129.5 (2), 127.6  
25 (4), 118.3 (3, d,  $J$  = 85 Hz), 63.4, 31.9, 26.8 (3), 25.3, 23.0, 22.4 (d,  $J$  = 3 Hz), 19.1; IR (film)  $\nu_{\text{max}}$  3394, 2928, 2856, 2359, 1438, 1111, 996, 743, 703, 689  $\text{cm}^{-1}$ ; ESI ( $\text{M} - \text{Br}^+$ ) 587.

A solution of **TBDPSphosphonium salt** (2.5783 g, 3.9 mmol, 1.1 eq) in anhydrous THF (25 mL) under  $\text{N}_2$  at  $-78^\circ\text{C}$  was treated  
30 with  $n\text{-BuLi}$  (2.0M, 3.5 mL, 7.0 mmol, 2 eq). The reaction mixture was warmed to  $25^\circ\text{C}$  and allowed to stir for 10 min. The reaction was recooled to  $-78^\circ\text{C}$  and treated with a solution of **CHO-alkene** (0.66 g, 3.5 mmol, 1 eq) in anhydrous THF (10 mL).

The reaction mixture was then warmed to 25 °C and stirred for 1 h before an aqueous solution of saturated  $\text{NH}_4\text{Cl}$  (60 mL) was added. The aqueous layer was extracted with EtOAc (3 x 60 mL) and the organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed (5  $\text{SiO}_2$ , 3 x 20 cm, 0-20% EtOAc/hexanes, gradient elution) to afford **alkene-alkene** (0.57 g, 33%) as a clear oil (mixture of E/Z isomers);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.67 - 7.57 (m, 4H), 7.45 - 7.33 (m, 7H), 7.24 - 7.09 (m, 3H), 6.63 - 6.35 (m, 2H), 10 6.14 - 5.61 (m, 2H), 3.70 - 3.58 (m, 2H), 2.21 - 2.06 (m, 4H), 1.64 - 1.23 (m, 8H), 1.04 - 1.01 (m, 9H), 0.94 - 0.80 (m, 3H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  136.6, 136.5, 135.5, 134.1, 133.2, 133.0, 132.8, 129.5, 129.1, 129.0, 128.0, 127.8, 127.6, 126.8, 126.2, 125.3, 63.7, 33.0, 32.2, 32.1, 32.0, 28.19, 28.15, 26.8, 15 26.0, 25.9, 25.7, 22.3, 19.2, 13.9; IR (film)  $\nu_{\text{max}}$  2929, 2857, 1473, 1428, 1111, 823, 740, 701  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  629.2226 ( $\text{C}_{34}\text{H}_{44}\text{OSi}$  + Cs $^+$  requires 629.2216).

**Alkene-alkene** (0.4311 g, 0.87 mmol, 1 eq) was combined with 10% Pd/C (0.33 g) under Ar. Absolute EtOH (8 mL) was added and the atmosphere was purged to  $\text{H}_2$ . The reaction was stirred at 25 °C for 24 h. The crude product was filtered through Celite to afford **alkane** (347.3 mg, 80%) as a clear oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.84 - 7.78 (m, 4H), 7.56 - 7.44 (m, 6H), 7.27 - 7.21 (m, 25 4H), 3.80 (t, 2H,  $J$  = 6.5 Hz), 2.73 (td, 4H,  $J$  = 7.9, 2.4 Hz), 1.70 (m, 6H), 1.58 - 1.42 (m, 10H), 1.19 (s, 9H), 1.02 (t, 3H,  $J$  = 7.0 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  140.5, 140.4, 135.6, 134.1, 129.5, 129.1, 127.6, 125.7, 63.9, 32.7, 32.6, 32.5, 31.7, 31.3, 29.50, 29.46, 26.9, 25.7, 22.6, 19.2, 14.1; IR (film)  $\nu_{\text{max}}$  3070, 30 2928, 2856, 1956, 1888, 1823, 1589, 1471, 1427, 1389, 1361, 1188, 1111, 1007, 939, 910, 823, 740, 701, 614  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  633.2525 ( $\text{C}_{34}\text{H}_{48}\text{OSi}$  + Cs $^+$  requires 633.2529).

A solution of **alkane** (325.7 mg, 0.65 mmol, 1 eq) in anhydrous THF (6 mL) under  $\text{N}_2$  was treated with TBAF (1M in THF,

1.3 mL, 1.3 mmol, 2 eq) and stirred at 25 °C for 1.5 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was

5 chromatographed (SiO<sub>2</sub>, 2.5 x 15 cm, 10-33% EtOAc/hexanes, gradient elution) to afford **alcohol** (142.7 mg, 84%) as a clear oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.18 - 7.11 (m, 4H), 3.64 (t, 2H, J = 6.6 Hz), 2.66 - 2.61 (m, 4H), 1.64 - 1.57 (m, 6H), 1.45 - 1.32 (m, 10H), 0.94 (t, 3H, J = 6.9 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)

10 δ 140.4, 140.2, 129.02, 128.98, 125.7, 125.6, 62.7, 32.6 (2), 32.5, 31.7, 31.22, 31.20, 29.5, 29.4, 25.6, 22.6, 14.0; IR (film)  $\nu_{\max}$  3340, 2928, 2856, 1489, 1463, 1055, 750 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 262.2308 (C<sub>18</sub>H<sub>30</sub>O +  $\cdot$  requires 262.2297).

A solution of **alcohol** (128.9 mg, 0.49 mmol, 1 eq) in

15 anhydrous DMF (1 mL) under N<sub>2</sub> was treated with PDC (0.91 g, 2.4 mmol, 5 eq) at 25 °C for 8 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 2.5 x 15 cm, 20 -

20 50% EtOAc/hexanes, gradient elution) to afford **acid** (88.1 mg, 65%) as a clear oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.16 - 7.10 (m, 4H), 2.61 (q, 4H, J = 7.2 Hz), 2.37 (t, 2H, J = 7.5 Hz), 1.70 (p, 2H, J = 7.7 Hz), 1.59 (septet, 4H, J = 7.7 Hz), 1.48 - 1.30 (m, 8H), 0.90 (t, 3H, J = 7.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ

25 180.3, 140.5, 140.0, 129.1, 129.0, 125.8, 125.7, 34.0, 32.7, 32.4, 31.7, 31.3, 30.9, 29.4, 29.1, 24.6, 22.6, 14.0; IR (film)  $\nu_{\max}$  2928, 2858, 2359, 1709, 1489, 1462, 1412, 1287, 1242, 941, 751 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 299.1976 (C<sub>18</sub>H<sub>28</sub>O<sub>2</sub> + Na $\cdot$  requires 299.1987).

30 A solution of **acid** (62.3 mg, 0.23 mmol, 1 eq) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (1.2 mL) under N<sub>2</sub> at 0 °C was treated with oxalyl chloride (2M in CH<sub>2</sub>Cl<sub>2</sub>, 0.34 mL, 0.68 mmol, 3 eq). The reaction mixture was warmed to 25 °C and stirred for 3 h before the solvent was removed in vacuo. The residue was cooled to 0 °C and treated



with excess concentrated  $\text{NH}_4\text{OH}$  (5 mL). Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 2.5 x 15 cm, 5 33-66% EtOAc/hexanes, gradient elution) to afford 6 (36.9 mg, 59%) as a white solid; mp 58 - 59 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.14 - 7.08 (m, 4H), 5.91 (br, 1H), 5.49 (br, 1H), 2.58 (q, 4H,  $J = 6.4$  Hz), 2.20 (t, 2H,  $J = 7.6$  Hz), 1.66 (hextet, 2H,  $J = 7.7$  Hz), 1.55 (hex, 4H,  $J = 8.4$  Hz), 1.45 - 1.28 (m, 8H), 0.88 (t, 10 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.7, 140.5, 140.0, 129.1, 129.0, 125.8, 125.7, 35.8, 32.6, 32.4, 31.7, 31.2, 30.9, 29.4, 29.2, 25.4, 22.6, 14.1; IR (film)  $\nu_{\text{max}}$  3366, 3195, 2925, 2855, 1664, 1629, 1491, 1465, 1412, 750  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  276.2329 ( $\text{C}_{18}\text{H}_{29}\text{ON} + \text{H}^+$  requires 276.2327).

15

**7 derivative as illustrated in Figure 7** Synthesized exactly as found in the general procedure prepared in a manner analogous to 6; all starting reagents were purchased from Aldrich, Acros, or Sigma: white solid; mp 95-96 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  20 7.06 (s, 4H), 5.58 (br, 1H), 5.40 (br, 1H), 2.54 (t, 4H,  $J = 7.8$  Hz), 2.18 (t, 2H,  $J = 7.6$  Hz), 1.57 (m, 6H), 1.35 - 1.25 (m, 12H), 0.86 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.6, 140.2, 139.7, 128.22 (2), 128.19 (2), 35.9, 35.5, 35.4, 31.8, 31.6, 31.3, 29.3, 29.2, 29.0, 28.9, 25.4, 22.7, 14.1; IR (film) 25  $\nu_{\text{max}}$  3388, 3187, 2923, 2851, 1645, 1514, 1463, 1416, 1121, 810  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  304.2651 ( $\text{C}_{20}\text{H}_{33}\text{ON} + \text{H}^+$  requires 304.2640).

**8 derivative as illustrated in Figure 7 and scheme shown on**  
30 **Figure 20.** Synthesized exactly as found in the general procedure prepared in a manner analogous to 6; all starting reagents were purchased from Aldrich, Acros, or Sigma: white solid; mp 87-88 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.16 (t, 1H,  $J =$

7.7 Hz), 6.96 (t, 3H,  $J = 6.6$  Hz), 5.66 (br, 1H), 5.42 (br, 1H), 2.55 (t, 4H,  $J = 7.8$  Hz), 2.19 (t, 2H,  $J = 6.0$  Hz), 1.60 (septet, 6H,  $J = 8.0$  Hz), 1.37 - 1.28 (m, 10H), 0.86 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.8, 142.8, 142.5, 128.5, 128.0, 125.6, 125.5, 35.92, 35.85, 35.81, 31.7, 31.5, 31.3, 29.02 (2), 29.00, 25.3, 22.5, 14.1; IR (film)  $\nu_{\text{max}}$  3352, 3191, 2925, 2854, 1660, 1630, 1466, 1409, 1341, 1311, 1253, 1202, 1138, 896, 777, 702  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  312.2312 ( $\text{C}_{19}\text{H}_{31}\text{ON} + \text{Na}^+$  requires 312.2303).

10

CH( $\text{CH}_3$ ) derivative as illustrated in Figure 8 and scheme shown on Figure 21. A fresh solution of LDA was prepared at  $-78^\circ\text{C}$  under Ar from anhydrous diisopropylamine (0.47 mL, 3.35 mmol, 2.2 eq) and n-BuLi (2.2M in hexanes, 1.4 mL, 3.1 mmol, 2 eq) in anhydrous THF (6 mL). A solution of methyl oleate (0.4556 g, 1.54 mmol, 1 eq) in THF (1 mL) was added dropwise at  $-78^\circ\text{C}$ . After 50 min of additional stirring, MeI (0.96 mL, 15.4 mmol, 10 eq) was added and the reaction mixture was allowed to warm to  $25^\circ\text{C}$  and stir for 12 h. Saturated aqueous  $\text{NH}_4\text{Cl}$  (30 mL) was added to the dark orange solution and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 2 x 15 cm, 0 - 5% EtOAc/hexanes, gradient elution) to afford  $\alpha$ -Me-ester (0.4276 g, 90%) as a clear oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  5.33 - 5.30 (m, 2H), 3.64 (s, 3H), 2.40 (s, 1H,  $J = 7.0$  Hz), 2.00 - 1.96 (m, 4H), 1.64 - 1.60 (m, 1H), 1.39 - 1.24 (m, 21H), 1.11 (d, 3H,  $J = 7.0$  Hz), 0.85 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  177.4, 130.0, 129.7, 51.4, 39.4, 33.8, 31.9, 29.8, 29.7, 29.5, 29.4, 29.3, 29.1, 27.2, 27.1, 22.7, 17.0, 14.1; IR (film)  $\nu_{\text{max}}$  2925, 2854, 1741, 1462, 1195, 1157, 723  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  443.1938 ( $\text{C}_{20}\text{H}_{38}\text{O}_2 + \text{Cs}^+$  requires 443.1926).

30

A solution of  $\alpha$ -Me-ester (150.5 mg, 0.48 mmol, 1 eq) in THF/MeOH/ $\text{H}_2\text{O}$  (3:1:1; 5 mL) was treated with LiOH (67.1 mg, 1.6

mmol, 3.3 eq) and stirred at 25 °C for 15 h. A second portion of LiOH (66.1 mg, 1.58 mmol, 3.3 eq) was added and the reaction was stirred for an additional 7 h. Aqueous HCl (1N, 30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 2 x 15 cm, 5 - 20% EtOAc/hexanes, gradient elution) to afford  $\alpha$ -Me-acid (111.6 mg, 78%) as a clear oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  5.37 - 5.29 (m, 2H), 2.43 (s, 1H, *J* = 6.9 Hz), 1.99 (m, 4H), 1.66 (p, 1H, *J* = 7.2 Hz), 1.43 - 1.25 (m, 21H), 1.15 (d, 3H, *J* = 7.0 Hz), 0.86 (t, 3H, *J* = 6.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  183.5, 130.0, 129.7, 39.4, 33.5, 31.9, 29.8, 29.7, 29.5, 29.4, 29.3, 29.1, 27.2, 27.14, 27.10, 22.7, 16.8, 14.1; IR (film)  $\nu_{\max}$  2925, 2854, 1708, 1466, 1291, 1239, 941, 723 cm<sup>-1</sup>; FABHRMS (NBA-NaI) *m/z* 297.2797 (C<sub>19</sub>H<sub>36</sub>O<sub>2</sub> + H<sup>+</sup> requires 297.2794).

A solution of  $\alpha$ -Me-acid (94.4 mg, 0.32 mmol, 1 eq) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (1.6 mL) under N<sub>2</sub> at 0 °C was treated with oxalyl chloride (2M in CH<sub>2</sub>Cl<sub>2</sub>, 0.48 mL, 0.96 mmol, 3 eq). The reaction mixture was warmed to 25 °C and stirred for 3 h before the solvent was removed in vacuo. The residue was cooled to 0 °C and treated with excess concentrated NH<sub>4</sub>OH (1 mL). Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The crude product was chromatographed (SiO<sub>2</sub>, 2 x 5 cm, 33 - 50% EtOAc/hexanes, gradient elution) to afford CH(CH<sub>3</sub>) (70.3 mg, 75%) as a white solid: mp 43-44 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  6.20 (br, 1H), 5.59 (br, 1H), 5.33 - 5.24 (m, 2H), 2.20 (s, 1H, *J* = 6.8 Hz), 1.98 - 1.93 (m, 4H), 1.61 - 1.54 (m, 1H), 1.36 - 1.22 (m, 21H), 1.10 (d, 3H, *J* = 6.9 Hz), 0.83 (t, 3H, *J* = 6.9 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  179.7, 129.9, 129.7, 40.8, 34.2, 31.8, 29.7, 29.6, 29.5, 29.2, 29.1, 27.3, 27.13, 27.08, 22.6, 17.7, 14.0; IR (film)  $\nu_{\max}$  3360, 3186, 2922, 2852, 1654, 1628, 1466, 1414, 1376, 1140, 722, 697 cm<sup>-1</sup>; FABHRMS (NBA) *m/z* 296.2959 (C<sub>19</sub>H<sub>31</sub>NO + H<sup>+</sup> requires 296.2953).

$C(CH_3)_2$  derivative as illustrated in Figure 8 and scheme shown on Figure 21: A fresh solution of LDA was prepared at  $-78\text{ }^\circ\text{C}$  under Ar from anhydrous diisopropylamine (0.20 mL, 1.4 mmol, 2.2 eq) and n-BuLi (2.2M in hexanes, 0.6 mL, 1.3 mmol, 2 eq) in anhydrous THF (2.6 mL). A solution of  $\alpha$ -Me-ester (0.2024 g, 0.65 mmol, 1 eq) in THF (0.4 mL) was added dropwise at  $-78\text{ }^\circ\text{C}$ . After 50 min of additional stirring, MeI (0.41 mL, 6.6 mmol, 10 eq) was added and the reaction mixture was allowed to warm to  $25\text{ }^\circ\text{C}$  and stir for 17 h. Saturated aqueous  $NH_4Cl$  (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried ( $Na_2SO_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $SiO_2$ , 2 x 8 cm, 0 - 5% EtOAc/hexanes, gradient elution) to afford  $\alpha,\alpha$ -diMe-ester (170.9 mg, 81%) as a clear oil;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  5.34 - 5.25 (m, 2H), 3.61 (s, 3H), 1.99 - 1.94 (m, 4H), 1.48 - 1.44 (m, 2H), 1.27 - 1.23 (m, 20H), 1.11 (s, 6H), 0.84 (t, 3H,  $J = 6.8$  Hz);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$  178.5, 129.9, 129.7, 51.5, 42.2, 40.8, 31.9, 30.0, 29.7, 29.6, 29.5, 29.3, 29.1, 27.14, 27.11, 25.1, 24.9, 22.6, 14.0; IR (film)  $\nu_{max}$  2926, 2854, 1736, 1464, 1192, 1152  $cm^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  325.3101 ( $C_{21}H_{40}O_2 + H^+$  requires 325.3107).

A solution of  $\alpha,\alpha$ -diMe-ester (157.6 mg, 0.49 mmol, 1 eq) in THF/MeOH/ $H_2O$  (3:1:1; 5 mL) was treated with LiOH (408.5 mg, 9.74 mmol, 20 eq) and stirred at  $25\text{ }^\circ\text{C}$  for 19 h. A second portion of LiOH (408.7 mg, 9.74 mmol, 20 eq) was added and the reaction was stirred for an additional 26 h. A third portion of LiOH (392.0 mg, 9.34 mmol, 19 eq) was added and the reaction was stirred for 47 h at  $25\text{ }^\circ\text{C}$  and 23 h at  $70\text{ }^\circ\text{C}$ . Aqueous HCl (1N, 30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried ( $Na_2SO_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $SiO_2$ , 2 x 8 cm, 5 - 50% EtOAc/hexanes, gradient elution) to afford  $\alpha,\alpha$ -diMe-acid (123.4 mg, 82%) as a clear oil;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  5.37 - 5.28 (m, 2H), 2.00 - 1.97 (m, 4H), 1.53

- 1.49 (m, 2H), 1.32 - 1.25 (m, 20H), 1.17 (s, 6H), 0.86 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  185.3, 130.0, 129.8, 42.1, 40.5, 31.9, 30.0, 29.8, 29.7, 29.5, 29.3, 29.1, 27.20, 27.16, 24.9, 24.8, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  2925, 2854, 1701, 1467, 1277, 1200, 938  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  333.2778 ( $\text{C}_{20}\text{H}_{38}\text{O}_2 + \text{Na}^+$  requires 333.2770).

A solution of  $\alpha,\alpha$ -diMe-acid (101.5 mg, 0.33 mmol, 1 eq) in anhydrous  $\text{CH}_2\text{Cl}_2$  (1.6 mL) under  $\text{N}_2$  at  $0^\circ\text{C}$  was treated with oxalyl chloride (2M in  $\text{CH}_2\text{Cl}_2$ , 0.49 mL, 0.98 mmol, 3 eq). The reaction mixture was warmed to  $25^\circ\text{C}$  and stirred for 3 h before the solvent was removed in vacuo. The residue was cooled to  $0^\circ\text{C}$  and treated with excess concentrated  $\text{NH}_4\text{OH}$  (1 mL). Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 x 30 mL). The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 2 x 5 cm, 33 - 50% EtOAc/hexanes, gradient elution) to afford  $\text{C}(\text{CH}_3)_2$  (90.5 mg, 89%) as a white solid: mp  $61\text{-}62^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.10 (br, 1H), 5.61 (br, 1H), 5.34 - 5.26 (m, 2H), 1.97 - 1.94 (m, 4H), 1.47 - 1.42 (m, 2H), 1.30 - 1.23 (m, 20H), 1.13 (s, 6H), 0.84 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  181.0, 129.9, 129.7, 41.9, 41.3, 31.8, 30.0, 29.70, 29.67, 29.5, 29.3, 29.1, 27.2, 27.1, 25.4, 24.7, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  3396, 3213, 3002, 2923, 2851, 1650, 1620, 1466, 1401, 1364, 1109  $\text{cm}^{-1}$ ; FABHRMS (NBA-CsI)  $m/z$  310.3115 ( $\text{C}_{20}\text{H}_{39}\text{NO} + \text{H}^+$  requires 310.3110).

"O" derivative as illustrated in Figure 8 and scheme shown on Figure 21: Commercially available from Aldrich.

"NH" derivative as illustrated in Figure 8 and scheme shown on Figure 21: Commercially available from Aldrich.

CH(SH) derivative as illustrated in Figure 8 and scheme shown in Figure 22: A solution of CH(SAc) (72.3 mg, 0.20 mmol, 1 eq) in

- 66 -

anhydrous  $\text{CH}_2\text{Cl}_2$  (4 mL) under  $\text{N}_2$  at  $-78^\circ\text{C}$  was treated with DIBAL (1M in toluene, 0.61 mL, 0.61 mmol, 3 eq). The reaction mixture was allowed to stir for 1.5 h before a second portion of DIBAL (1M in toluene, 0.61 mL, 0.61 mmol, 3 eq) was added. After 30 min, the reaction was warmed to  $25^\circ\text{C}$  before MeOH (2 mL) and aqueous HCl (5%, 30 mL) were added. The reaction mixture was allowed to stir for 15 min. The aqueous layers were extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 30 mL). The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 1 x 8 cm, 20 - 50% EtOAc/hexanes, gradient elution) to afford **CH(SH)** (31.5 mg, 49%) as a clear oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.22 (br, 1H), 5.46 (br, 1H), 5.37 - 5.28 (m, 2H), 3.28 (q, 1H,  $J = 6.5$  Hz), 2.00 - 1.91 (m, 4H), 1.74 - 1.64 (m, 1H), 1.29 - 1.24 (m, 21H), 0.86 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  175.0, 130.1, 129.7, 43.0, 35.5, 32.0, 29.8, 29.6, 29.5, 29.3, 29.0, 27.2, 27.1, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  3363, 3186, 2923, 2853, 2362, 1662, 1458  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  336.2341 ( $\text{C}_{18}\text{H}_{35}\text{ONS} + \text{Na}^+$  requires 336.2337).

**CH(SAc): derivative as illustrated in Figure 8 and scheme shown in Figure 22:** A solution of  $\text{Ph}_3\text{P}$  (182.2 mg, 0.69 mmol, 2 eq) in anhydrous THF (3.5 mL) under  $\text{N}_2$  at  $0^\circ\text{C}$  was treated with DEAD (110  $\mu\text{L}$ , 0.70 mmol, 2 eq) and allowed to stir for 30 min. Thiolacetic acid (50  $\mu\text{L}$ , 0.70 mmol, 2 eq) was added followed by 2-hydroxy-9Z-octadecenamide (**CH(OH)**; 103.0 mg, 0.35 mmol, 1 eq) in THF (2.2 mL). The reaction mixture was stirred at  $0^\circ\text{C}$  for 2 h before the addition of saturated aqueous  $\text{NaHCO}_3$  (30 mL). The aqueous layers were extracted with EtOAc (3 x 30 mL). The organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated in vacuo. The crude product was chromatographed ( $\text{SiO}_2$ , 2 x 10 cm, 10 - 50% EtOAc/hexanes, gradient elution) to afford **CH(SAc)** (96.7 mg, 79%) as a clear oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.12 (br, 1H), 5.63 (br, 1H), 5.35 - 5.26 (m, 2H), 3.91 (t, 1H,  $J = 7.6$  Hz), 2.35 (s, 3H), 2.02 - 1.90 (m, 5H), 1.67 - 1.58 (m, 1H),



1.42 - 1.24 (m, 20H), 0.85 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  196.6, 173.4, 130.0, 129.6, 45.6, 31.9, 30.3, 29.9, 29.7, 29.6, 29.5, 29.3, 29.1, 29.0, 27.2, 27.1, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  3448, 3352, 3186, 2923, 2853, 1682, 1457, 1396, 1353, 1259, 1116, 954  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  378.2433 ( $\text{C}_{20}\text{H}_{37}\text{O}_2\text{NS} + \text{Na}^+$  requires 378.2443).

CH(OH)derivative as illustrated in Figure 8: prepared as previously described (JACS, 1996, 118, 5938-5945).

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CHCl derivative as illustrated in Figure 8: prepared as previously described (JACS, 1996, 118, 5938-5945).

C(=O) derivative as illustrated in Figure 8: prepared as previously described (JACS, 1996, 118, 5938-5945).

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C(=O)CH<sub>2</sub>derivative as illustrated in Figure 8: prepared as previously described (JACS, 1996, 118, 5938-5945)

20 Synthesis of 1-Bromo-10Z-nonadecen-2-one as shown in Figure 3.

1-Diazo-10Z-nonadecen-2-one (15, 92 mg, 0.30 mmol, 1 equiv; synthesis described herein) was treated with a solution of EtOAc saturated with HBr (1.5 mL). The reaction mixture was stirred at 25 °C for 25 min. The solvent was removed by a stream of  $\text{N}_2$  and the crude product was chromatographed ( $\text{SiO}_2$ , 2 to 8 cm, 0-5% EtOAc-hexanes gradient elution) to afford 41 (99 mg, 92%) as clear oil:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.37-5.28 (m, 2H), 3.86 (s, 2H), 2.62 (t, 2H,  $J = 7.4$  Hz), 2.02-1.96 (m, 4H), 1.59 (m, 2H), 1.28-1.24 (m, 20H), 0.86 (t, 3H,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  202.0, 130.0, 129.7, 39.8, 34.3, 31.9, 29.7, 29.6, 29.5, 29.3(2), 29.2, 29.04, 28.99, 27.2, 27.1, 23.8, 22.7, 14.1; IR (film)  $\nu_{\text{max}}$  2924, 2853, 1718, 1560, 1262, 1103, 1026  $\text{cm}^{-1}$ ; FABHRMS (NBA-NaI)  $m/z$  381.1760 ( $\text{C}_{19}\text{H}_{35}\text{BrO} + \text{Na}^+$  requires 381.1769).

30

**Synthese of 9Z-Docosen-1-ol as illustrated in Figure 3. 9-**

Bromononanol was protected (TBDPSCl, 1.1 equiv, Et<sub>3</sub>N, 1.2 equiv, DMAP, 0.3 equiv, 25 °C, 2.5 h) and treated with PPh<sub>3</sub> (1.3 equiv, CH<sub>3</sub>CN, 85 °C, 12 h) to generate a phosphonium salt (285 mg, 0.42 mmol, 1 equiv) that was dissolved in anhydrous THF (2.6 mL) under Ar at -78 °C. The solution was treated dropwise with KHMDS (0.5 M in toluene, 0.85 mL, 0.43 mmol, 1 equiv) and was stirred for 40 min at -78 °C before tridecanal (100 mL, 0.42 mmol, 1 equiv) was added. The reaction was warmed to 25 °C and allowed to stir 1 h before saturated aqueous NH<sub>4</sub>Cl (30 mL) was added. The aqueous layer was extracted with EtOAc (3 to 30 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The crude product was chromatographed. Next, a solution of the above product (128 mg, 0.23 mmol, 1 equiv) in THF (2.2 mL) under N<sub>2</sub> was treated with Bu<sub>4</sub>NF (1 M in THF, 0.46 mL, 0.46 mmol, 2 equiv) and stirred at 25 °C for 2.5 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 to 30 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The crude product was chromatographed (SiO<sub>2</sub>, 2 to 5 cm, 5-33% EtOAc-hexane gradient elution) to afford 72 (51 mg, 69%) as a white film: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.36-5.29 (m, 2H), 3.62 (t, 2H, J = 6.6 Hz), 2.02-1.97 (m, 4H), 1.54 (m, 2H), 1.28-1.24 (m, 30H), 0.86 (t, 3H, J = 6.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 129.9, 129.8, 63.1, 32.8, 31.9, 29.73 (2), 29.67 (2), 29.64 (3), 29.6, 29.5, 29.39, 29.35, 29.31, 29.2, 27.2, 25.7, 22.7, 14.1; IR (film) ν<sub>max</sub> 3328, 2923, 2852, 1457, 1055 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 325.3475 (C<sub>22</sub>H<sub>44</sub>O + H<sup>+</sup> requires 325.3470).

30

**Synthesis of 4-[(6R\*,1R\*)-6-Butoxymethyl-3-cyclohexenyl-1-methoxy]butyramide (trans isomer to compound #5, Figure 7) - note, this compound is not shown in Figure 7, but is claimed)**  
Butyraldehyde (2.0 mL, 22.2 mmol, 3 equiv) and pyridinium p-

toluenesulfonate (191 mg, 0.76 mmol, 0.1 equiv) were added to a solution of commercially available [1(R\*)6(R\*)-hydroxymethyl-3-cyclohexenyl] (1.07 g, 7.5 mmol, 1 equiv; Aldrich) in anhydrous benzene (25 mL) under N<sub>2</sub> atmosphere. The reaction was heated to reflux with a Dean-Stark trap for 1.5 h. Saturated aqueous NaHCO<sub>3</sub> (60 mL) was added and the aqueous layer was extracted with EtOAc (3 to 60 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The crude product was chromatographed (SiO<sub>2</sub>, 3 to 15 cm, 0-10% EtOAc-hexane gradient elution) to afford 147 (1.12 g, 76%) as a clear oil (diastereomer with lower R<sub>f</sub>): <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.59 (s, 2H), 4.70 (t, 1H, J = 5.6 Hz), 3.78 (d, J = 6.2 Hz, 1H), 3.75 (d, J = 6.1 Hz, 1H), 3.55 (d, J = 2.7 Hz, 1H), 3.52 (d, J = 2.4 Hz, 1H), 2.15-2.02 (m, 6H), 1.54-1.48 (m, 2H), 1.34 (m, 2H), 0.89 (t, 3H, J = 7.3 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 125.2(2), 102.1, 68.4 (2), 37.0 (2), 36.7, 26.1 (2), 18.0, 13.9; IR (film) ν<sub>max</sub> 3024, 2959, 2931, 2873, 1732, 1462, 1378, 1271, 1143, 991, 825 cm<sup>-1</sup>; FABHRMS opens up acetal. Next, A solution of the above material (98 mg, 0.50 mmol, 1 equiv) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) under N<sub>2</sub> atmosphere at 0 °C was treated with DIBAL (1 M in toluene, 2.5 mL, 2.5 mmol, 5 equiv) for 15 min. The reaction was warmed to 25 °C for 3.5 h. The reaction mixture was recooled to 0 °C before the addition of CH<sub>3</sub>OH (2 mL) and 1 N aqueous HCl (30 mL). After 15 min, the aqueous layer was extracted with EtOAc (3 to 30 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The crude product was chromatographed (SiO<sub>2</sub>, 2 to 8 cm, 10-20% EtOAc-hexane gradient elution) to afford 148 (86.7 mg, 88%) as a clear oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.60-5.54 (m, 2H), 3.61-3.28 (m, 7H), 2.16-1.92 (m, 6H), 1.53 (m, 2H), 1.34 (hex, 2H, J = 7.6 Hz), 0.88 (t, 3H, J = 7.4 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 125.7, 125.3, 72.3, 71.2, 64.4, 38.2, 35.1, 31.5, 28.0, 26.2, 19.3, 13.8; IR (film) ν<sub>max</sub> 3395, 3022, 2957, 2571, 1463, 1439, 1378, 1112 1037, 622 cm<sup>-1</sup>; FABHRMS (NBA-NaI)

m/z 199.1694 ( $C_{12}H_{22}O_2 + H^+$  requires 199.1698). Next, A solution of the above compound (91 mg, 0.46 mmol, 1 equiv) in anhydrous DMF (2.5 mL) under  $N_2$  atmosphere at 0 °C was treated with NaH (39 mg, 0.99 mmol, 2 equiv). The reaction was allowed  
5 to stir at 25 °C for 15 min and then was recooled to 0 °C. 4-Bromobutyronitrile (230 mL, 2.3 mmol, 5 equiv) was added dropwise and the reaction mixture was heated to 90 °C for 24 h and 140 °C for 17 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3  $\times$  30 mL). The combined  
10 organic layers were dried ( $Na_2SO_4$ ), filtered, and concentrated under reduced pressure. The crude product was chromatographed ( $SiO_2$ , 2  $\times$  10 cm, 2-20% EtOAc-hexane gradient elution) to afford 149 (25 mg, 20%) as a clear oil:  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  5.60 (s, 2H), 3.52-3.25 (m, 8H), 2.44 (t, 2H,  $J = 7.1$  Hz), 2.15-2.08  
15 (m, 4H), 1.91-1.84 (m, 4H), 1.52 (m, 2H), 1.35 (m, 2H), 0.89 (t, 3H,  $J = 7.4$  Hz);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$  125.7, 125.4, 119.6, 72.0, 71.6, 70.9, 68.2, 34.7, 34.6, 31.8, 27.0, 26.8, 25.8, 19.4, 14.1, 13.9; IR (film)  $\nu_{max}$  3020, 2930, 2866, 1726, 1463, 1438, 1377, 1113, 662  $cm^{-1}$ ; FABHRMS (NBA-NaI) m/z  
20 266.2125 ( $C_{16}H_{27}NO_2 + H^+$  requires 266.2120). Also isolated was 150 (28 mg, 27%) as a clear oil:  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  8.05 (s, 1H), 5.65-5.57 (m, 2H), [4.22 and 4.20] [d, 1H,  $J = 5.8$  Hz], [4.10 (d,  $J = 8.6$  Hz) and 4.08 (d,  $J = 9.0$  Hz)] [1H], 3.43-3.28 (m, 4H), 2.27-2.09 (m, 4H), 1.96-1.86 (m, 2H), 1.52 (m, 2H),  
25 1.34 (m, 2H), 0.89 (t, 3H,  $J = 7.3$  Hz);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$  161.2, 125.8, 124.9, 71.5, 71.0, 64.9, 34.5, 33.8, 31.8, 26.8, 26.5, 19.3, 13.9; IR (film)  $\nu_{max}$  3024, 2958, 2928, 2862, 1727, 1466, 1439, 1378, 1167, 1113, 663  $cm^{-1}$ ; FABHRMS (NBA-NaI) m/z 227.1655 ( $C_{13}H_{22}O_3 + H^+$  requires 227.1647). A solution of  
30 the above compound (320 mg, 1.61 mmol, 1 equiv) in anhydrous DMF (8 mL) under  $N_2$  atmosphere at 0 °C was treated with NaH (77 mg, 1.93 mmol, 1.2 equiv) for 15 min. A solution of 151 (0.95 g, 2.31 mmol, 1.4 equiv) in DMF (3 mL) was added dropwise and the reaction mixture was allowed to stir at 25 °C for 17 h.  $Bu_4NI$

(0.60 g, 1 equiv) was added and the reaction was stirred 7 h, and heated to 60 °C for 19 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 to 30 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The crude product was chromatographed (SiO<sub>2</sub>, 2 to 10 cm, 0-20% EtOAc-hexane gradient elution) to afford the product (123 mg, 15%) as a clear oil. Next, a solution of the above compound (111 mg, 0.22 mmol, 1 equiv) in anhydrous THF (2 mL) under N<sub>2</sub> atmosphere at 25 °C was treated with Bu<sub>4</sub>NF (1 M in THF, 0.44 mL, 0.44 mmol, 2 equiv) for 3 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3 to 30 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The crude product was chromatographed. Next, A solution of the above compound (33 mg, 0.12 mmol, 1 equiv) in anhydrous DMF (0.6 mL) under N<sub>2</sub> atmosphere was treated with PDC (0.23 g, 0.61 mmol, 5 equiv). The reaction was stirred at 25 °C for 4.5 h, filtered through Celite, and concentrated under reduced pressure. Next, A solution of the above compound (41 mg, 0.14 mmol, 1 equiv) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL) under N<sub>2</sub> at 0 °C was treated with oxalyl chloride (2 M in CH<sub>2</sub>Cl<sub>2</sub>, 0.21 mL, 0.42 mmol, 3 equiv). The reaction mixture was warmed to 25 °C and stirred for 3 h in the dark before the solvent was removed under reduced pressure. The residue was cooled to 0 °C, diluted with EtOAc, and treated with excess NH<sub>4</sub>OH (1 mL). Water (30 mL) was added and extracted with EtOAc (3 to 30 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The crude product was chromatographed (SiO<sub>2</sub>, 1 to 10 cm, 50-100% EtOAc-hexane gradient elution) to afford product (21 mg, 52%) as a clear oil. Next a solution of the above cyclohexene compound (21 mg, 0.074 mmol, 1 equiv) was combined with 10% Pd/C (11 mg) under Ar atmosphere. Absolute EtOH (1 mL) was added and the atmosphere was replaced with H<sub>2</sub>. The reaction was stirred at 25 °C for 21 h. A second portion of

10% Pd/C (20 mg) was added and the reaction was stirred an additional 16 h. The crude mixture was filtered through Celite and the solvent removed under reduced pressure to afford product (19 mg, 89%) as a clear oil;

5 Finally, 4-[(6R\*,1R\*)-6-Butoxymethyl-3-cyclohexenyl-1-methoxy]butyramide was synthesized using a solution of the above compound; 31 mg, 0.12 mmol, 1 equiv) and powdered KOH (62 mg, 1.1 mmol, 9 equiv) in t-butanol (0.12 mL) was heated to 100 °C for 30 min. The crude reaction was cooled to 25 °C, diluted  
10 with brine (2 mL), and extracted with EtOAc (5 to 2 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The crude product was chromatographed (SiO<sub>2</sub>, 1 to 6 cm, 50-80% EtOAc-hexane gradient elution) to afford final product (25 mg, 76%) as a clear oil:  
15 <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.79 (br, 1H), 5.60 (s, 2H), 5.31 (br, 1H), 3.46-3.25 (m, 8H), 2.32 (t, 2H, J = 7.2 Hz), 2.16-2.06 (m, 4H), 1.93-1.85 (m, 4H), 1.55-1.48 (m, 2H), 1.38-1.28 (m, 2H), 0.89 (t, 3H, J = 7.3 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 175.4, 125.7, 125.5, 71.8, 71.4, 70.9, 69.8, 34.7, 34.5, 32.8, 31.8,  
20 27.1, 26.8, 25.4, 19.4, 13.9; IR (film) ν<sub>max</sub> 3341, 3203, 3022, 2929, 2860, 1667, 1435, 1404, 1377, 1110, 661 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 284.2235 (C<sub>16</sub>H<sub>29</sub>NO<sub>3</sub> + H<sup>+</sup> requires 284.2226).

**Synthesis of 4-[(1R\*,2R\*)-6-Butoxymethyl-3-cyclohexyl-1-methoxy]butyramide (trans isomer to compound #5, Figure 7) -**  
25 **note, this compound is not shown in Figure 7, but is claimed) .**  
Cyclohexene (19 mg, 0.068 mmol, 1 equiv; *vide supra*) was combined with 10% Pd/C (21 mg) under Ar atmosphere. Absolute EtOH (1 mL) was added and the atmosphere was replaced with H<sub>2</sub>.  
30 The reaction was stirred at 25 °C for 24 h. The crude mixture was filtered through Celite and the solvent removed under reduced pressure to afford 164 (18 mg, 91%) as a clear oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.83 (br, 1H), 5.34 (br, 1H), 3.44-3.25 (m, 8H), 2.32 (t, 2H, J = 7.1 Hz), 1.95-1.85 (m, 4H), 1.55-1.29



(m, 12H), 0.89 (t, 3H, J = 7.4 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 175.4, 71.7, 71.3, 70.8, 69.8, 37.5, 37.2, 32.8, 31.8, 26.9, 26.6, 25.5, 23.7, 23.5, 19.4, 13.9; IR (film) ν<sub>max</sub> 3351, 3194, 2916, 2855, 1667, 1614, 1446, 1377, 1261, 1104, 667 cm<sup>-1</sup>;  
5 FABHRMS (NBA-NaI) m/z 308.2194 (C<sub>16</sub>H<sub>31</sub>NO<sub>3</sub> + Na<sup>+</sup> requires 308.2202).

**Synthesis of methanesulfonic Acid 7Z-Hexadecenyl Ester,**  
claimed but not shown, this compound is similar to compounds as  
illustrated in Figure 3. A solution of 7Z-hexadecenol (42 mg,  
10 0.18 mmol, 1 equiv) and anhydrous Et<sub>3</sub>N (55 mL, 0.39 mmol, 2.2  
equiv) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL) under N<sub>2</sub> atmosphere was  
treated with MsCl (30 mL, 0.39 mmol, 2.2 equiv). The reaction  
was stirred at 25 °C for 4 h before the addition of water (2  
mL). The aqueous layer was extracted with EtOAc (5 to 2 mL) and  
15 the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and  
concentrated under reduced pressure. The crude product was  
chromatographed (SiO<sub>2</sub>, 2 to 8 cm, 10-20% EtOAc-hexane gradient  
elution) to afford 175 (56 mg, 100%) as a clear oil: <sup>1</sup>H NMR  
(CDCl<sub>3</sub>, 400 MHz) δ 5.37-5.27 (m, 2H), 4.19 (t, 2H, J = 6.6 Hz),  
20 2.97 (s, 3H), 1.99 (m, 4H), 1.72 (m, 2H), 1.41-1.24 (m, 18H),  
0.85 (t, 3H, J = 6.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 130.2,  
129.3, 70.1, 37.3, 31.8, 29.7, 29.5 (2), 29.3 (2), 29.1, 28.6,  
27.2, 26.9, 25.3, 22.6, 14.1; IR (film) ν<sub>max</sub> 2924, 2854, 1463,  
1356, 1176, 954, 816, 719 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 341.2135  
25 (C<sub>17</sub>H<sub>34</sub>O<sub>3</sub>S + Na<sup>+</sup> requires 341.2126).

**Synthesis of 1-Azido-7Z-hexadecene,** claimed but not shown,  
this compound is similar to compounds as illustrated in Figure  
3. A solution of methanesulfonic Acid 7Z-Hexadecenyl Ester (49  
mg, 0.15 mmol, 1 equiv; *vide supra*) in anhydrous DMF (1.0 mL)  
30 under N<sub>2</sub> atmosphere was treated with NaN<sub>3</sub> (21 mg, 0.32 mmol, 2.2  
equiv). The reaction was stirred at 25 °C for 23 h before the  
addition of water (2 mL). The aqueous layer was extracted with  
EtOAc (5 to 2 mL) and the combined organic layers were dried  
(Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure.

The crude product was chromatographed (SiO<sub>2</sub>, 2 to 5 cm, 0-2% EtOAc-hexane gradient elution) to afford 176 (37 mg, 90%) as a clear oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.38-5.28 (m, 2H), 3.24 (t, 2H, J = 7.0 Hz), 2.00 (m, 4H), 1.58 (m, 2H), 1.39-1.25 (m, 18H), 0.86 (t, 3H, J = 6.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 130.2, 129.5, 51.5, 31.9, 29.7, 29.6, 29.5, 29.3 (2), 28.80, 28.76, 27.2, 27.0, 26.6, 22.7, 14.1; IR (film) ν<sub>max</sub> 2925, 2854, 2094, 1460, 1260 cm<sup>-1</sup>; ESMS (NBA-NaI) m/z 288 (M + Na<sup>+</sup>).

**Synthesis of 7Z-Hexadecenamine, claimed but not shown,**  
10 this compound is similar to compounds as illustrated in Figure 3. A solution of azide (57 mg, 0.22 mmol, 1 equiv; *vide supra*) in anhydrous Et<sub>2</sub>O (1.0 mL) under N<sub>2</sub> atmosphere was cooled to 0 °C and treated with LiAlH<sub>4</sub> (19 mg, 0.50 mmol, 2.3 equiv). The reaction was allowed to stir at 25 °C for 20 min. The mixture  
15 was then recooled to 0 °C and saturated aqueous Na<sub>2</sub>SO<sub>4</sub> was added until all excess LiAlH<sub>4</sub> was quenched. Solid Na<sub>2</sub>SO<sub>4</sub> was added, the mixture was filtered, and the solvent removed under reduced pressure to afford 177 (44 mg, 86%) as a clear oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.36-5.28 (m, 2H), 2.67 (br, 2H), 2.00-1.96 (m, 4H), 1.45-1.24 (m, 20H), 0.85 (t, 3H, J = 6.7 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 130.0, 129.7, 42.2, 31.9, 29.8, 29.7 (2), 29.5, 29.3 (2), 29.1, 27.2, 27.1, 26.8, 22.7, 14.1; IR (film) ν<sub>max</sub> 2923, 2853, 1570, 1464, 1312, 818, 724 cm<sup>-1</sup>; FABHRMS (NBA-NaI) m/z 240.2698 (C<sub>16</sub>H<sub>33</sub>N + H<sup>+</sup> requires 240.2691).

25 **Materials** The fatty acid primary amides (Figure 2) were prepared by treating the acid chlorides, generated from the corresponding carboxylic acid and oxalyl chloride, with aqueous NH<sub>4</sub>OH according the procedures in Cravatt et al. (1996) *J. Am. Chem. Soc.* 118, 580-590; Patterson et al. (1996) *J. Am. Chem. Soc.* 118, 5938-5945). Many of the fatty acids were commercially  
30 available (Sigma, Aldrich, Fluka), and the remainder were synthesized as detailed *vide infra*. Most agents in Figure 3 were prepared by condensing oleoyl chloride with the appropriate amine or alcohol following procedures in Cravatt et al. (1996)

*J. Am. Chem. Soc.* **118**, 580-590; Patterson et al. (1996) *J. Am. Chem. Soc.* **118**, 5938-5945; Roe et al. *J. Am. Chem. Soc.* **71**, 2215-2218; Roe et al. (1952) *J. Am. Chem. Soc.* **74**, 3442-3443. The remaining agents in Figure 3 are commercially available (Sigma, Pfaltz & Bauer) or are prepared *vide infra*. The ethanolamides (Figure 4) were similarly prepared or purchased (Pfaltz & Bauer). The synthesis of the 18-hydroxyoleamide was described in Cravatt et al. (1996) *J. Am. Chem. Soc.* **118**, 580-590 and standard transformations following protocols detailed therein were used to prepare the remaining agents in Figure 5. *N*-Oleoyl glycine (Figure 6) was prepared by coupling glycine ethyl ester to oleic acid with EDCI (Cravatt et al. (1996) *J. Am. Chem. Soc.* **118**, 580-590; Patterson et al. (1996) *J. Am. Chem. Soc.* **118**, 5938-5945) followed by sequential transformation to the carboxylic acid (LiOH) and glycinamide (EDCI, NH<sub>4</sub>OH;). *N*-Oleoyl sarcosine was purchased (Pfaltz and Bauer) and converted to its ethyl ester by coupling with EtOH (DCC). The agents in Figure 7 were prepared as described in Cravatt et al. (1996) *J. Am. Chem. Soc.* **118**, 580-590; Simmons et al. (1959) *J. Am. Chem. Soc.* **81**, 4256-4264) or by a series of Wittig couplings to the appropriately substituted *o*, *m*, or *p*-cyanobenzaldehyde. Substitutions at the  $\alpha$ -carbon (Figure 8) were installed by treating the enolate of oleic acid or methyl oleate (generated by LDA) with an appropriate electrophile as previously detailed in Patterson et al. (1996) *J. Am. Chem. Soc.* **118**, 5938-5945). The primary carbamate and urethanes were prepared from the corresponding alcohols or amines (HCl, NaOCN).

#### Cell culture

Rat glial cells (Suter et al., 1987) obtained from Dr. Trosko's laboratory were cultured in standard plastic tissue cultureware in Richter's Improved Minimal Essential Medium (Irvine Scientific, Santa Ana, CA), supplemented with 10% fetal

calf serum and 50  $\mu$ g/ml gentamicin sulfate, and incubated in a humidified atmosphere of 95% air/5% CO<sub>2</sub> at 37°C. The cells were passaged by trypsinization and used at passages 4-8. BHK (baby hamster kidney) cells that were stably transfected with a  $\beta$ 1 connexin cDNA (Kumar et al., 1995) were cultured in DMEM medium supplemented with 5% fetal calf serum and 50  $\mu$ g/ml gentamicin sulfate, and incubated in a humidified atmosphere of 95% air/5% CO<sub>2</sub> at 37° C. In order to induce  $\beta$ 1 connexin expression in the BHK cells, 100  $\mu$ M zinc acetate was added to culture medium for 8-18 hours when the cell culture was about 90% confluent.

#### Gap junction dye coupling assays

Gap junctional communication in glial cell and BHK/ $\beta$ 1 cell cultures was assayed by microinjection of 5% Lucifer yellow CH dye in 0.1 M LiCl solution, and quantitated by determining the number of directly adjacent, neighboring cells that received dye (dye-coupling). Micropipettes were loaded with the dye solution by backfilling. Cells were visualized using a Nikon Diaphot inverted phase contrast/ epifluorescent microscope, and impaled with dye-filled micropipettes using an Eppendorf microinjector (model 5246). Five minutes after dye-injection, the transfer of dye to directly adjacent cells was determined using epifluorescent illumination. For each treatment condition, ten cells were microinjected in each of three dishes, the total number of dye-coupled neighboring cells was calculated and compared to determine the % dye-coupling. For scrape loading measurements, Lucifer yellow CH (0.05% dye in PBS) was loaded intracellularly by cutting or scraping cells in the monolayer with a sharp knife. The dye solution was left in the dish for 90 seconds, then the solution was discarded, and the dish subsequently washed with PBS. The cells were examined for dye transfer with an inverted epifluorescence microscope, and the degree of communication was assessed by determining the extent of Lucifer yellow transfer into contiguous cells.

### Gap junction electrical coupling assay

Junctional conductance was measured using double whole cell patch recording performed on pairs of rat glial cells as described (Miller et al., (1992) J Mem. Biol. 128, 91-102). with  
5 a pipette solution of (in mM): Kaspertate 160, EGTA 10, CaCl<sub>2</sub> 2, HEPES 10 (pH 7.2), ATP 4. The external solution contained (in mM): NaCl 160, KCl 4.5, CaCl<sub>2</sub> 2, MgCl<sub>2</sub> 1, HEPES 10, pH 7.4. Both cells were held at -40 mV and pulses to -20 mV were alternately applied to each cell. Holding currents were  
10 subtracted in the records shown. Cells that were examined were generally in contact with other cells. The electrical conductance was calculated as the junctional current divided by 20 mV. All dye-coupling and conductance studies were performed at room temperature.

15

### Calcium wave images

Rat glial cells were loaded with 5  $\mu$ M Fluo-3/AM (Calbiochem) in Hank's balanced salt solution containing 25 mM HEPES buffer (HBSS/HEPES) for 1 hour, at which point the loading  
20 buffer was exchanged for new HBSS/HEPES buffer. The cell cultures were then left at room temperature for at least 30 minutes. Mechanical stimulation of a single cell was performed as follows: a glass micropipette (tip diameter of approximately 0.5  $\mu$ m) was micro-manipulated downward on to a single cell  
25 causing a transient deformation of the cell membrane. The calcium image was then examined with an inverted fluorescence microscope, and photographed with a digital fluorescence microscope (excitation = 506 nm, emission = 526 nm). The degree of calcium wave propagation was quantitated by counting at  
30 different time points the number of transmitting cells in one linear direction away from the stimulated cell. Junctional dye transfer rates were simultaneously examined by microinjection of Lucifer yellow CH in the same dishes; the methods for dye transfer assay was described in assay procedures, gap junction

dye coupling assays. For calcium wave and dye transfer studies, the drug was preincubated with the glial cells for 10 minutes, and the drug was left in the experimental solution throughout the examination. All measurements were performed at room  
5 temperature.

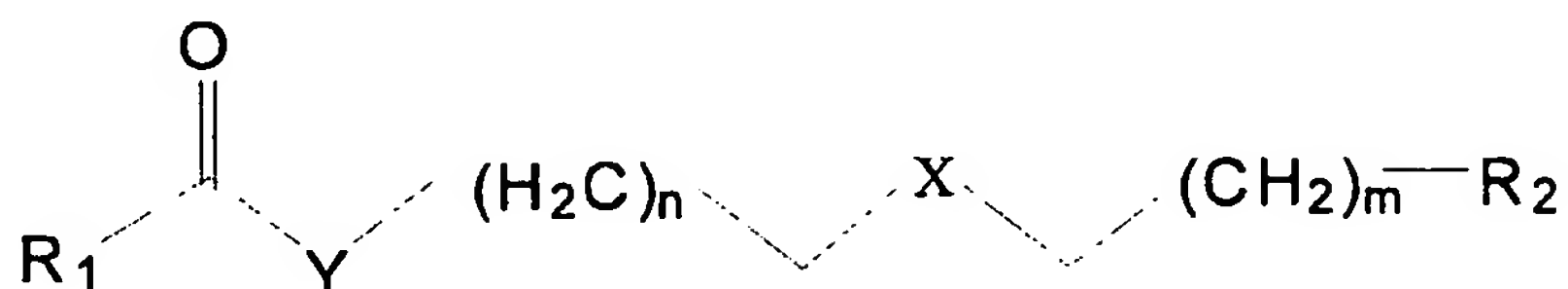
#### **Immunoblotting**

Plasma membrane fractions containing gap junctions were obtained after hypotonic alkali extraction of the glial cells.  
10 The extracted protein was dissolved in 2% SDS and the total protein was determined using the Bio-Rad DC Protein Assay kit (Bio-Rad Corp.). 10  $\mu$ g of protein was electrophoresed by 10% SDS-PAGE, and subsequently blotted electrophoretically on to Immobilon-P membranes (Millipore Corp.). Gap junction protein  
15 was detected using anti- $\alpha$ 1 connexin polyclonal rabbit antibodies, and the HRP/Chemiluminescence detection kit (Amersham Corp.) following the manufacturer's instructions.



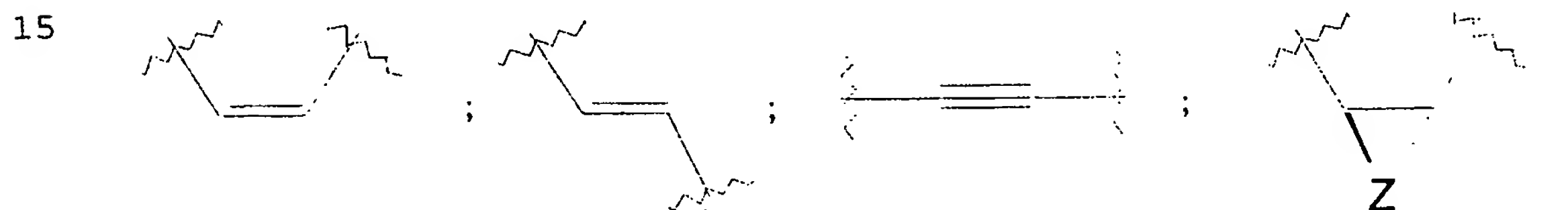
What is claimed is:

1. A compound having oleamide agonist activity for inhibiting gap junction-mediated chemical and electrical transmission in glial cells represented by the structure:



10 wherein:

X is a diradical selected from the group represented by the following structures:



wherein Z is a radical selected from the group consisting of:

20 -CH<sub>2</sub> and O;

Y is a diradical selected from the group consisting of: -CH<sub>2</sub>-, -CH(CH<sub>3</sub>)-, -C(CH<sub>3</sub>)<sub>2</sub>-, -O-, -NH-, -CH(SH)-, -CHSAc)-, -CH(OH)-, -CHCl-, -C(=O)-, -C(=O)CH<sub>2</sub>-, -CH<sub>2</sub>NHC(=O)-, and -CH<sub>2</sub>N(CH<sub>3</sub>)C(=O)-.

R<sub>1</sub> is a radical selected from the group consisting of:

hydrogen, -NH<sub>2</sub>, OH, MeNH-, Me<sub>2</sub>N-, EtNH-, Et<sub>2</sub>N-, CH<sub>2</sub>=CHCH<sub>2</sub>NH-, n-propyl-NH-, i-propyl-NH-, cyclopropyl-NH-, i-propyl-NMe-, butyl-NH-, pyrrolidine-, phenyl-NH-, phenyl(CH<sub>2</sub>)<sub>3</sub>NH-, HONH-, MeONMe-, NH<sub>2</sub>NH-, CH<sub>3</sub>O-, CH<sub>3</sub>CH<sub>2</sub>O-, CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>O-, Me<sub>2</sub>CHCH<sub>2</sub>O-, H-, CF<sub>3</sub>-, BrCH<sub>2</sub>-, ClCH<sub>2</sub>-, N<sub>2</sub>CH-, HOCH<sub>2</sub>CH<sub>2</sub>NH-, (HOCH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N-, HOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH- and HOCH<sub>2</sub>CH(OAc)CH<sub>2</sub>O-;

$R_2$  is a radical selected from the group consisting of:  $-CH_3$ ,  $-(CH_2)_2CH_3$ ,  $-(CH_2)_4CH_3$ ,  $-(CH_2)_6CH_3$ ,  $-CH_2OCH_3$ ,  $-CH_2OH$ ,  $-CONH_2$  and  $-CO_2H$ ;

5      $n$  is an integer from 0 to 15;  $m$  is an integer from 0 to 15  
with the requirement that the sum of  $n+m$  is an integer from  
11 to 15;

with the following provisos:

10

if  $Y$  is  $CH_2$ ,  $n$  is 4,  $m$  is 7, and  $R_2$  is  $CH_3$ , then  $R_1$  cannot  
be a radical selected from the group consisting of  $-CF_3$   
and hydrogen;

15

if  $Y$  is  $CH_2$ ,  $n$  is 5,  $m$  is 7, and  $R_2$  is  $CH_3$ , then  $R_1$  cannot  
be a radical selected from the group consisting of  $-$   
 $CF_3$ ,  $-CH_2Cl$ ,  $-NHOH$ ,  $-C(O)NH_2$ ,  $-CN_2$ , and  $-C(O)OEt$ ;

20

if  $Y$  is  $CHCl$ ,  $n$  is 4,  $m$  is 7, and  $R_2$  is  $CH_3$ , then  $R_1$   
cannot be  $NH_2$ ;

if  $Y$  is  $CH(OH)$ ,  $n$  is 4,  $m$  is 7, and  $R_2$  is  $CH_3$ , then  $R_1$   
cannot be  $NH_2$ ;

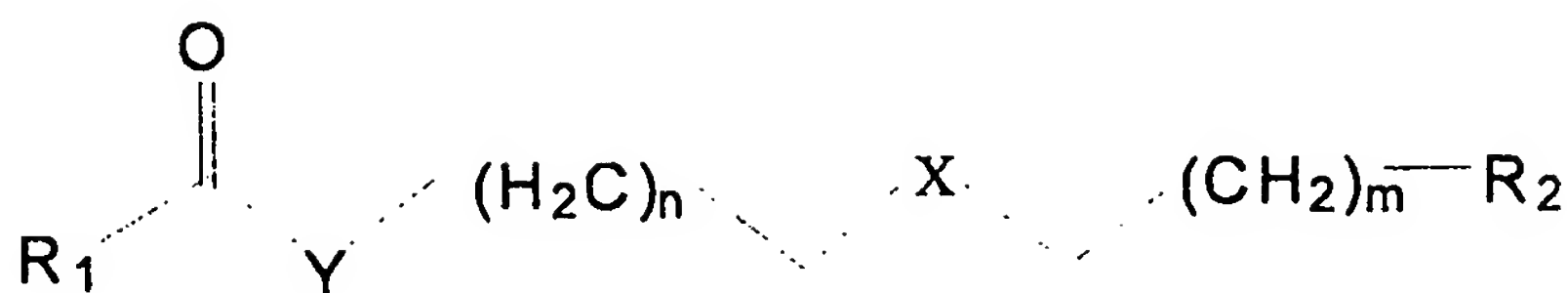
25

if  $Y$  is  $C(=O)$ ,  $n$  is 4,  $m$  is 7, and  $R_2$  is  $CH_3$ , then  $R_1$   
cannot be  $NH_2$  and  $CH_3CH_2O-$ ;

if  $Y$  is  $CH_2$ ,  $4 \leq n \leq 9$ ,  $4 \leq m \leq 7$ , and  $R_2$  is  $CH_3$ , then  $R_1$   
cannot be  $NH_2$  and  $OH$ .

30

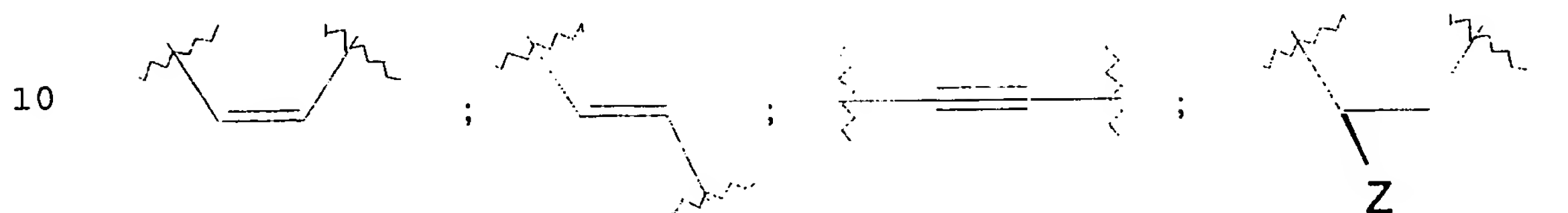
2. A method for inhibiting gap junction mediated chemical and  
electrical transmission in glial cells by contact with an  
oleamide agonist represented by the structure:



wherein:

5

X is a diradical selected from the group represented by the following structures:



wherein Z is a radical selected from the group consisting of:  
-CH<sub>2</sub> and O;

15

Y is a diradical selected from the group consisting of: -CH<sub>2</sub>-, -CH(CH<sub>3</sub>)-, -C(CH<sub>3</sub>)<sub>2</sub>-, -O-, -NH-, -CH(SH)-, -CHSAc)-, -CH(OH)-, -CHCl-, -C(=O)-, -C(=O)CH<sub>2</sub>-, -CH<sub>2</sub>NHC(=O)-, and -CH<sub>2</sub>N(CH<sub>3</sub>)C(=O)-.

20

R<sub>1</sub> is a radical selected from the group consisting of:  
hydrogen, -NH<sub>2</sub>, OH, MeNH-, Me<sub>2</sub>N-, EtNH-, Et<sub>2</sub>N-, CH<sub>2</sub>=CHCH<sub>2</sub>NH-,  
n-propyl-NH-, i-propyl-NH-, cyclopropyl-NH-, i-propyl-NMe-,  
butyl-NH-, pyrrolidine-, phenyl-NH-, phenyl(CH<sub>2</sub>)<sub>3</sub>NH-, HONH-,  
25 MeONMe-, NH<sub>2</sub>NH-, CH<sub>3</sub>O-, CH<sub>3</sub>CH<sub>2</sub>O-, CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>O-, Me<sub>2</sub>CHCH<sub>2</sub>O-, H-,  
CF<sub>3</sub>-, BrCH<sub>2</sub>-, ClCH<sub>2</sub>-, N<sub>2</sub>CH-, HOCH<sub>2</sub>CH<sub>2</sub>NH-, (HOCH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>N-,  
HOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH- and HOCH<sub>2</sub>CH(OAc)CH<sub>2</sub>O-;

25

30

R<sub>2</sub> is a radical selected from the group consisting of: -CH<sub>3</sub>, -  
(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>, -(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>, -(CH<sub>2</sub>)<sub>6</sub>CH<sub>3</sub>, -CH<sub>2</sub>OCH<sub>3</sub>, -CH<sub>2</sub>OH, -CONH<sub>2</sub> and  
-CO<sub>2</sub>H;

n is an integer from 0 to 15; m is an integer from 0 to 15  
with the requirement that the sum of n+m is an integer from

11 to 15;

with the following provisos:

5           if Y is CH<sub>2</sub>, n is 4, m is 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub> cannot  
          be a radical selected from the group consisting of -CF<sub>3</sub>  
          and hydrogen;

          if Y is CH<sub>2</sub>, n is 5, m is 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub> cannot  
10          be a radical selected from the group consisting of -  
          CF<sub>3</sub>, -CH<sub>2</sub>Cl, -NHOH, -C(O)NH<sub>2</sub>, -CN<sub>2</sub>, and -C(O)OEt;

          if Y is CHCl, n is 4, m is 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub>  
          cannot be NH<sub>2</sub>;

15           if Y is CH(OH), n is 4, m is 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub>  
          cannot be NH<sub>2</sub>;

          if Y is C(=O), n is 4, m is 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub>  
20          cannot be NH<sub>2</sub> and CH<sub>3</sub>CH<sub>2</sub>O-;

          if Y is CH<sub>2</sub>, 4 ≤ n ≤ 9, 4 ≤ m ≤ 7, and R<sub>2</sub> is CH<sub>3</sub>, then R<sub>1</sub>  
          cannot be NH<sub>2</sub> and OH.

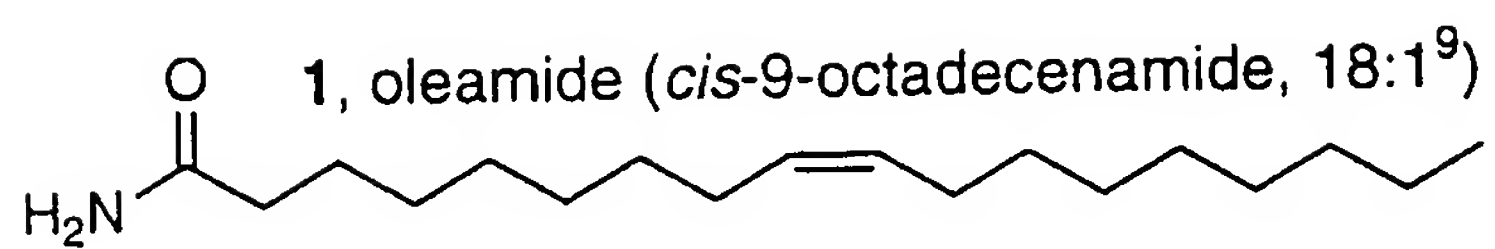


FIG. 1

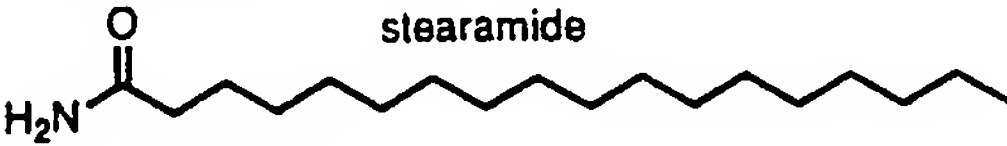
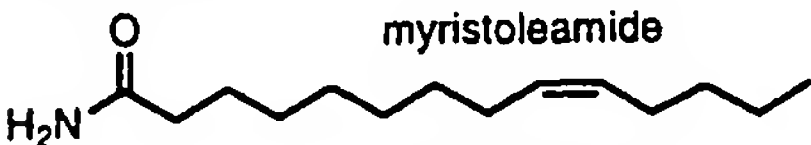
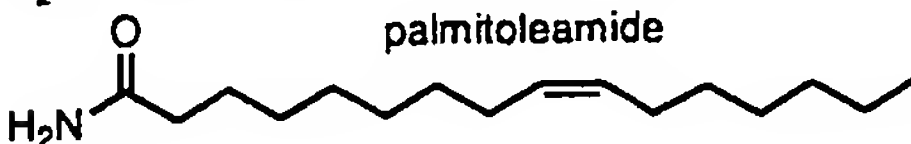
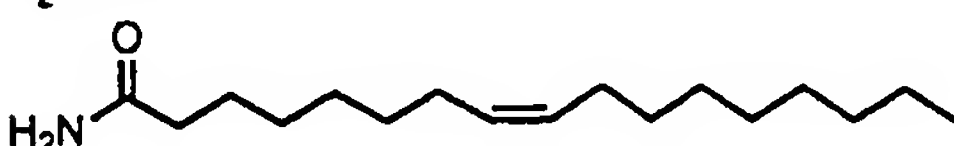
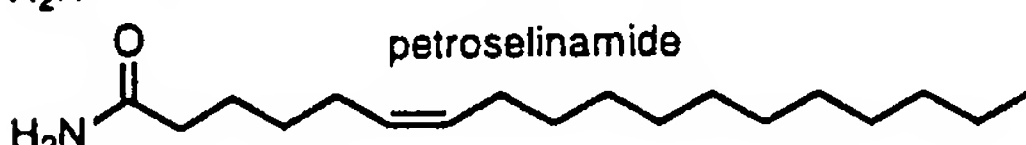
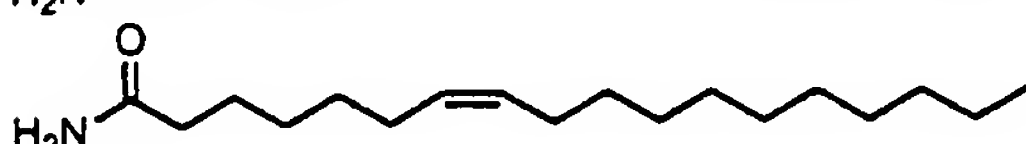


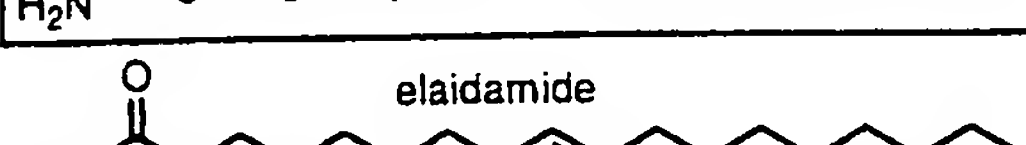

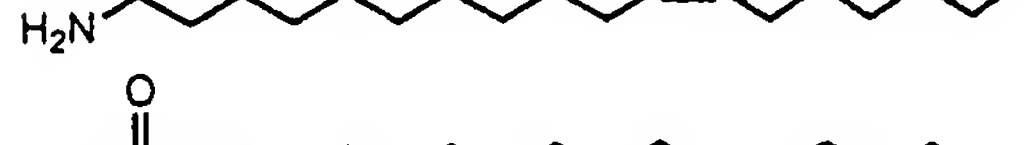
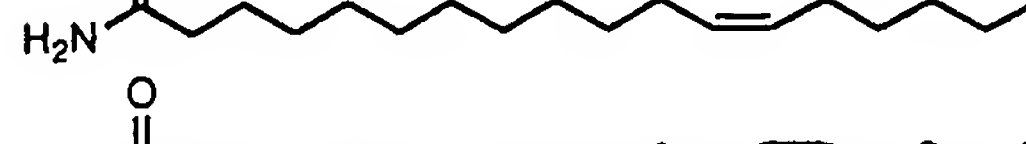
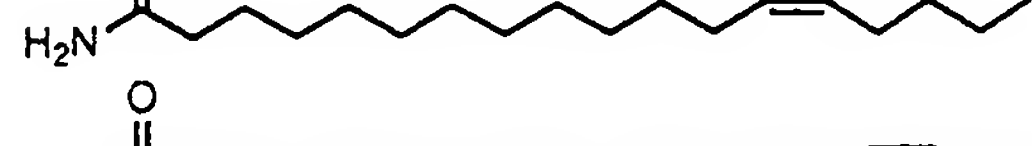
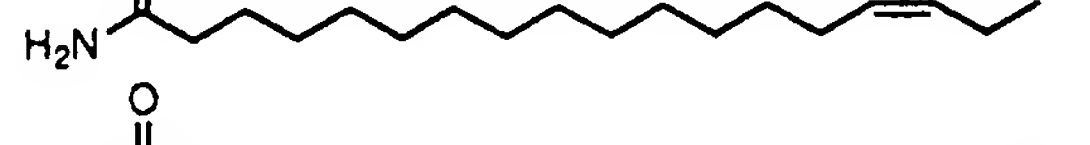
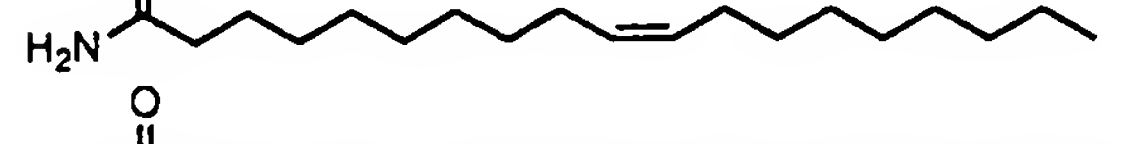
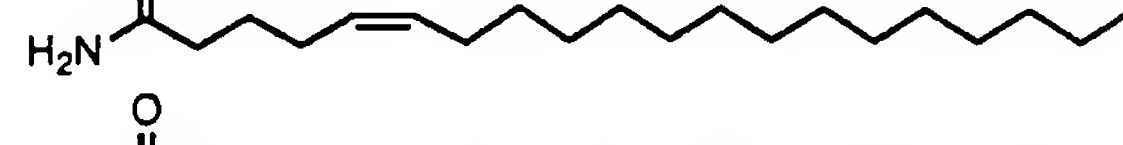
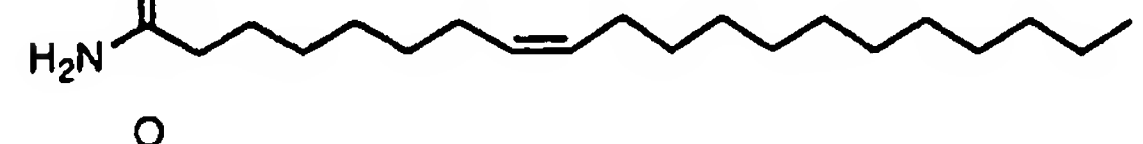
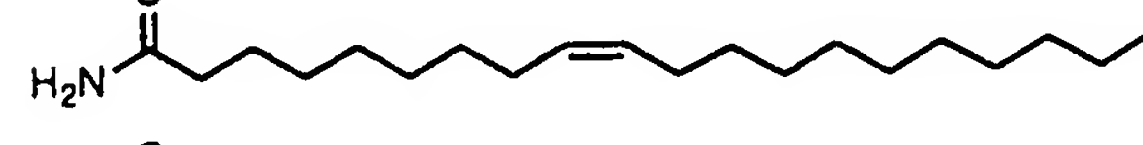
structure	notation <sup>a</sup>	% inhibition <sup>b</sup>		
		100 μM	50 μM	20 μM
no double bond				
 stearamide	18:0	nd <sup>c</sup>	0%	nd <sup>d</sup>
one double bond				
 myristoleamide	14:1 <sup>9</sup>	0%	0%	nd
 palmitoleamide	16:1 <sup>9</sup>	100%	100%	100%
 heptacosamide	17:1 <sup>8</sup>	nd	100%	100%
 petroselinamide	18:1 <sup>6</sup>	nd	100%	100%
 eicosenamide	18:1 <sup>7</sup>	nd	100%	100%
 nervonamide	18:1 <sup>8</sup>	100%	100%	30%
 oleamide	18:1 <sup>9</sup>	100%	100%	100%
 elaidamide	18:1 <sup>9-trans</sup>	0%	0%	0%
 vaccenamide	18:1 <sup>11</sup>	100%	25%	0%
 gaduamide	18:1 <sup>12</sup>	nd	90-100%	nd
 heneicosamide	18:1 <sup>13</sup>	nd	75-90%	nd
 docosamide	18:1 <sup>15</sup>	nd	0%	nd
 tricosamide	19:1 <sup>10</sup>	nd	100%	nd
 tetracosamide	20:1 <sup>5</sup>	nd	0%	nd
 pentacosamide	20:1 <sup>8</sup>	nd	95-100%	nd
 hexacosamide	20:1 <sup>9</sup>	100%	100%	0%
 heptacosamide	20:1 <sup>11</sup>	100%	100%	nd

FIG. 2A



3 / 22

structure	notation <sup>a</sup>	% inhibition <sup>b</sup>		
		100 $\mu$ M	50 $\mu$ M	20 $\mu$ M
	20:1 <sup>13</sup>	0%	0%	nd
	22:1 <sup>9</sup>	100%	100%	0%
erucamide	22:1 <sup>13</sup>	0%	0%	nd
	24:1 <sup>9</sup>	100%	100%	0%
nervonamide	24:1 <sup>15</sup>	0%	0%	nd
<i>two double bonds</i>				
linoleamide	18:2 <sup>9,12</sup>	nd	100%	10%
linoelaidamide	18:2 <sup>9,12-trans</sup>	75%	25%	nd
	20:2 <sup>11,14</sup>	nd	90%	20%
<i>three double bonds</i>				
γ-linolenamide	18:3 <sup>6,9,12</sup>	nd	100%	15%
α-linolenamide	18:3 <sup>9,12,15</sup>	0%	0%	nd
	20:3 <sup>8,11,14</sup>	nd	0%	nd
	20:3 <sup>11,14,17</sup>	nd	0%	nd
<i>four double bonds</i>				
arachidonamide	20:4 <sup>5,8,11,14</sup>	100%	100%	90-100%
<i>five double bonds</i>				
eicosapentaenamide	20:5 <sup>5,8,11,14,17</sup>	nd	100%	nd
<i>six double bonds</i>				
docosahexaenamide	22:6 <sup>4,7,10,13,16,19</sup>		0%	nd

FIG. 2B

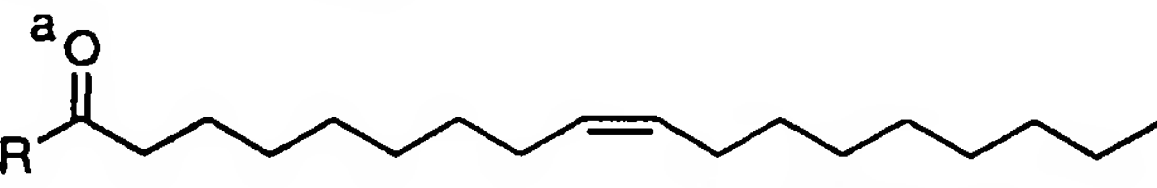



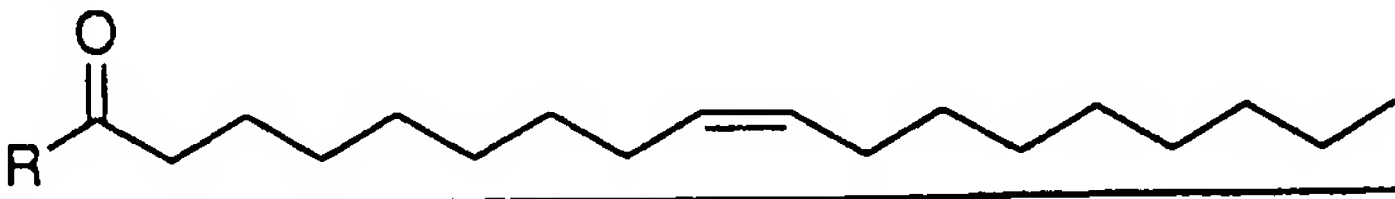
			
agent (R)	% inhibition <sup>a</sup>		
	100 $\mu$ M	50 $\mu$ M	20 $\mu$ M
NH <sub>2</sub> (oleamide)	100%	100%	100%
OH (oleic acid)	0%	0%	0%
MeNH	nd <sup>b</sup>	100%	70-80%
Me <sub>2</sub> N	nd	100%	70-85%
EtNH	nd	100%	50-60%
Et <sub>2</sub> N	nd	100%	15-25%
CH <sub>2</sub> =CHCH <sub>2</sub> NH	nd	100%	nd
PrNH	nd	90-100%	50-70%
<i>i</i> -PrNH	nd	100%	nd
 -NH	nd	100%	nd
<i>i</i> -PrNMe	nd	100%	nd
BuNH	nd	100%	nd
 N	nd	100% <sup>c</sup>	0% <sup>d</sup>
PhNH	nd	0%	nd
Ph(CH <sub>2</sub> ) <sub>3</sub> NH	nd	0%	nd
HONH	nd	100% <sup>c</sup>	0% <sup>d</sup>
MeONMe	nd	100% <sup>c</sup>	100% <sup>c,d</sup>
NH <sub>2</sub> NH	nd	0% <sup>e</sup>	nd
CH <sub>3</sub> O (methyl oleate)	0%	0%	nd
EtO (ethyl oleate)	nd	0%	nd
Me <sub>2</sub> CHCH <sub>2</sub> O	nd	0%	nd
H (oleyl aldehyde)	nd	100%	70-90%
CF <sub>3</sub>	nd	100% <sup>c</sup>	100% <sup>c,d,f</sup>
BrCH <sub>2</sub>	nd	15%	nd
ClCH <sub>2</sub>	nd	0%	nd
N <sub>2</sub> CH	nd	100%	nd
			
CH <sub>2</sub> OH (oleyl alcohol)	80-100%	15%	nd
CH <sub>2</sub> OAc (oleyl acetate)	nd	0%	nd
CH <sub>2</sub> NH <sub>2</sub> (oleyl amine)	100%	100%	90-100%
CH <sub>2</sub> NHCONH <sub>2</sub>	nd	10%	nd
CH(OMe) <sub>2</sub> (oleyl aldehyde dimethyl acetal)	nd	0%	nd
CoA-SCO	nd	0%	nd

FIG. 3

5 / 22

			
agent (R)	% inhibition <sup>a</sup>		
	100 $\mu$ M	50 $\mu$ M	20 $\mu$ M
NH <sub>2</sub> (oleamide)	100%	100%	100%
HOCH <sub>2</sub> CH <sub>2</sub> NH	100%	100%	0%
(HOCH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N	nd <sup>b</sup>	8%	nd
HOCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> NH	nd	100%	nd
HOCH <sub>2</sub> CH(OAc)CH <sub>2</sub> O <sup>c</sup>	50%	0%	0%

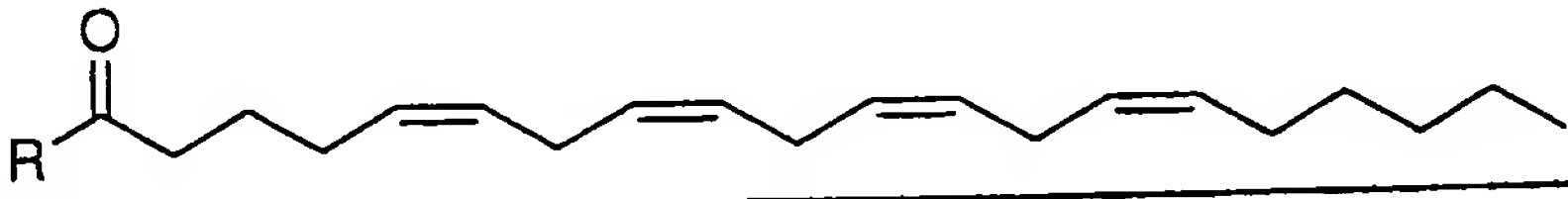
			
agent (R)	% inhibition <sup>a</sup>		
	100 $\mu$ M	50 $\mu$ M	20 $\mu$ M
NH <sub>2</sub>	100%	100%	90-100%
HOCH <sub>2</sub> CH <sub>2</sub> NH	100%	100%	35%
(HOCH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N	nd	0%	nd

FIG. 4

6 / 22

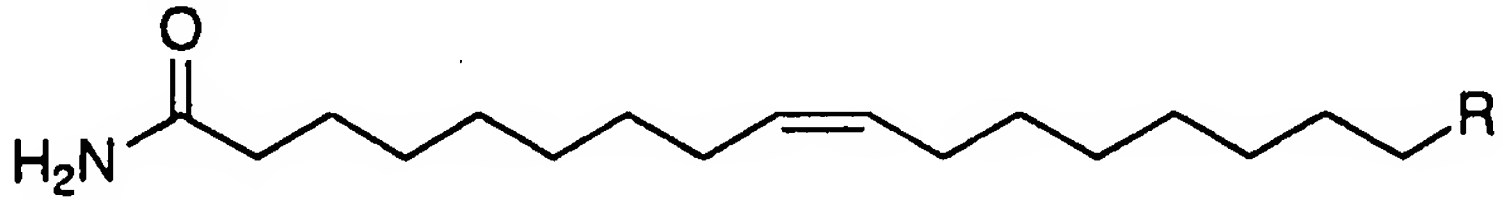
			
agent (R)	% inhibition <sup>a</sup>		
	100 $\mu$ M	50 $\mu$ M	20 $\mu$ M
CH <sub>3</sub> (oleamide)	100%	100%	100%
(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	100%	100%	0%
(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	100%	100%	0%
(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	100%	100%	0%
CH <sub>2</sub> OCH <sub>3</sub>	nd <sup>b</sup>	100%	nd
CH <sub>2</sub> OH	nd	8%	nd
CONH <sub>2</sub>	nd	0%	nd
CO <sub>2</sub> H	nd	0%	nd

FIG. 5

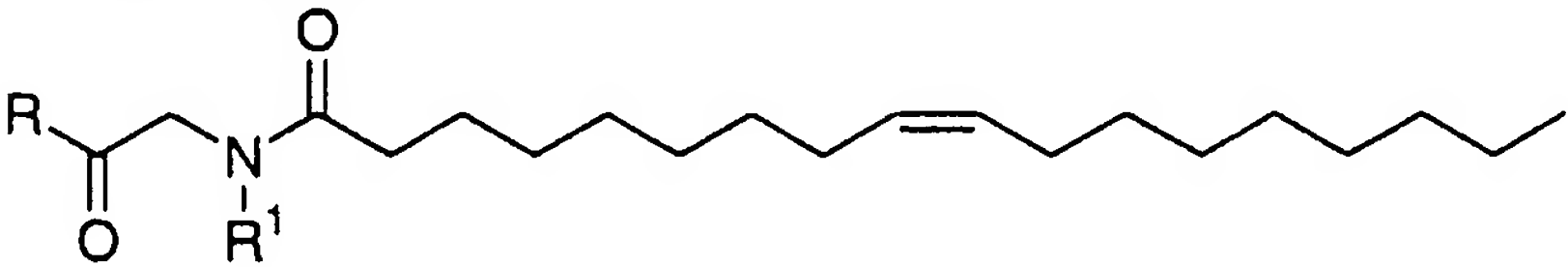
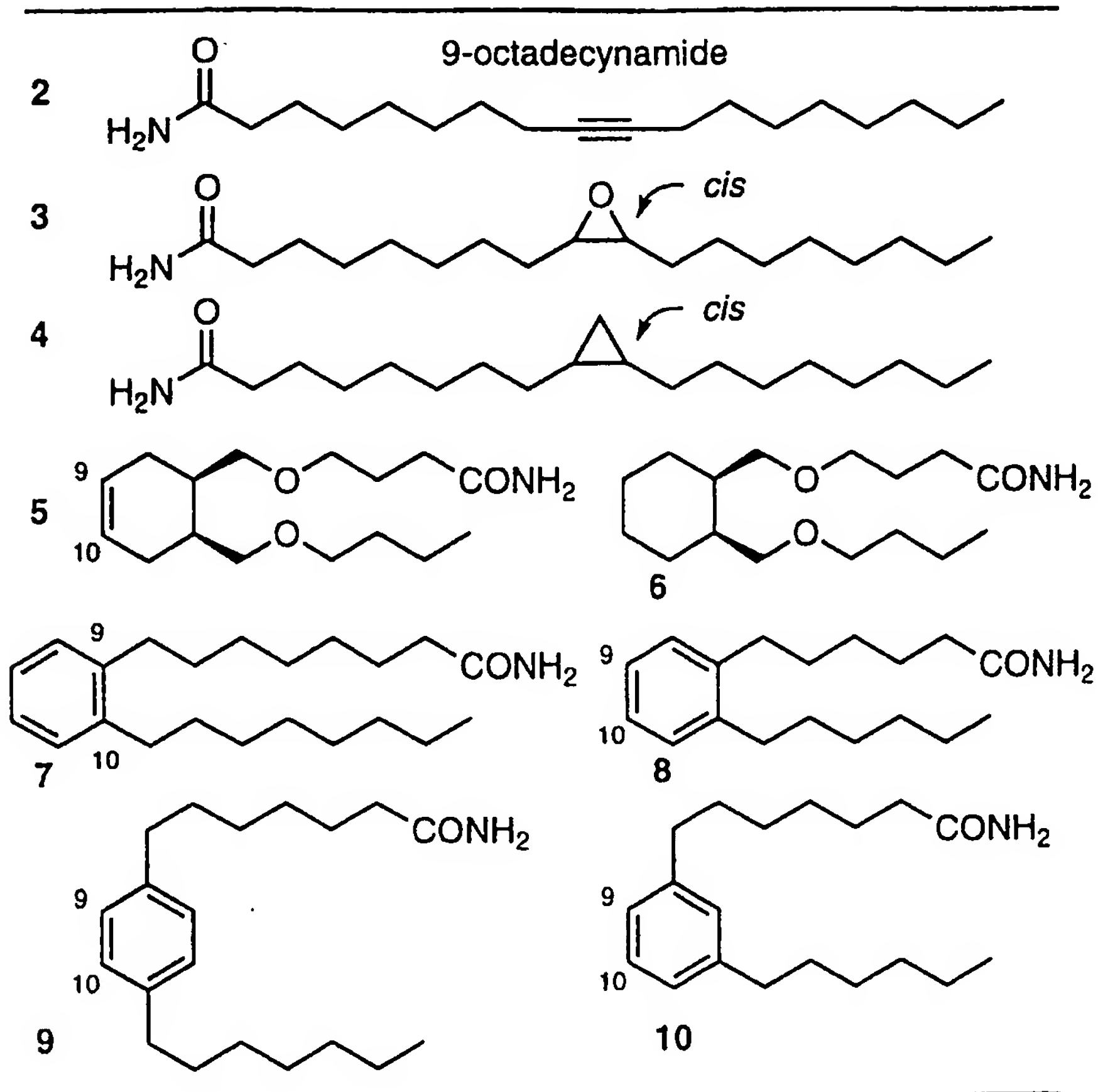
				
agent		% inhibition <sup>a</sup>		
R	R <sup>1</sup>	100 $\mu$ M	50 $\mu$ M	20 $\mu$ M
OH	H	nd	0%	nd <sup>b</sup>
NH <sub>2</sub>	H	nd	0% <sup>c</sup>	nd
OEt	H	nd	90-100%	nd
OH	Me	100%	100%	70-80%
OEt	Me	nd	90-100%	nd

FIG. 6



agent	% inhibition <sup>a</sup>		
	100 μM	50 μM	20 μM
1 (oleamide)	100%	100%	100%
2	nd <sup>b</sup>	90-100%	75-100%
3	0% <sup>c</sup>	0% <sup>c</sup>	0%
4	100%	75%	50-70%
cis-5	nd	60-70%	nd
cis-6	nd	20-30%	nd
trans-5	nd	100%	5%
trans-6	70%	0%	nd
7	100%	80-90%	80-100%
8	nd	90-100%	85-100%
9	nd	90-100%	0%
10	100%	0%	nd

FIG. 7


			
agent (X)	% inhibition <sup>a</sup>		
	100 μM	50 μM	20 μM
CH <sub>2</sub> (oleamide)	100%	100%	100%
CH(CH <sub>3</sub> )	nd <sup>b</sup>	100%	nd
C(CH <sub>3</sub> ) <sub>2</sub>	nd	100%	nd
O	nd	100%	nd
NH	nd	100%	nd
CH(SH)	0% <sup>c</sup>	0%	nd
CH(SAc)	nd	100%	nd
CH(OH)	nd	100%	80-100% <sup>d</sup>
CHCl	nd	100%	85-100% <sup>d</sup>
C(=O)	nd	100%	5-15% <sup>d</sup>
C(=O)CH <sub>2</sub>	nd	100%	20-40% <sup>d</sup>

FIG. 8



Treatment	Number of cells participating in wave ( in one direction)			Junction dye-coupling (%)
	2 sec	4 sec	8 sec	16 sec
Control	1.16±0.37	2.48±0.63	6.74±0.93	11.26±1.34
Oleamide (50 µM)	1.13±0.34	2.39±0.56	6.55±0.99	10.77±2.25
Anandamide (50 µM)	1.09±0.30	2.39±0.67	6.58±1.09	11.06±1.31
18β-GA (40 µM)	0.06±0.03	0.06±0.03	0.06±0.03	0.06±0.03
Heptanol (3 mM)	0.03±0.02	0.03±0.02	0.03±0.02	0.03±0.02
Suramin (200 µM)	0.90±0.30	0.94±0.33	0.94±0.33	0.94±0.33
Oleamide+Suramin	0.83±0.38	0.86±0.35	0.86±0.35	0.86±0.35

The number of cells participating in calcium wave propagation was quantified by counting the number of responding cells in one direction from the stimulated cell. The time points indicated above were analyzed after the initiation of calcium signaling; the responding cell numbers are expressed as Mean ± SD. Gap junction dye transfer was expressed as dye coupling percentage (Mean ± SD) which is described in Experimental Procedures. Each treatment was performed in 3 dishes, and 10 individual fields were examined in each dish, N=30.

FIG. 9

10 / 22

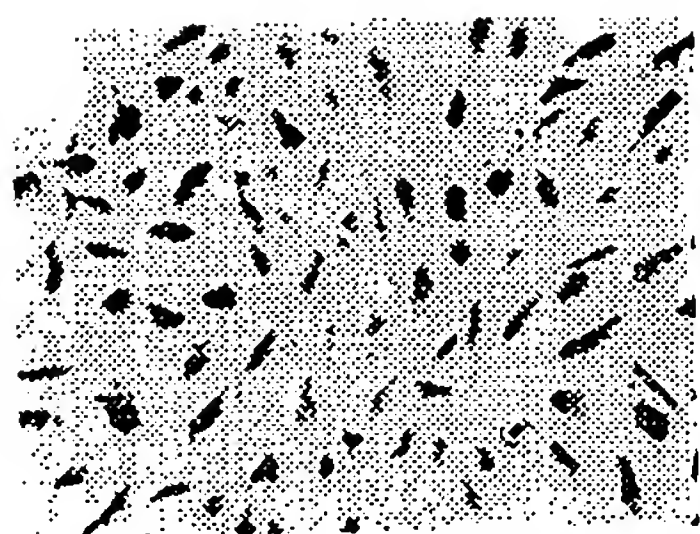


FIG. 10A



FIG. 10B



FIG. 10C

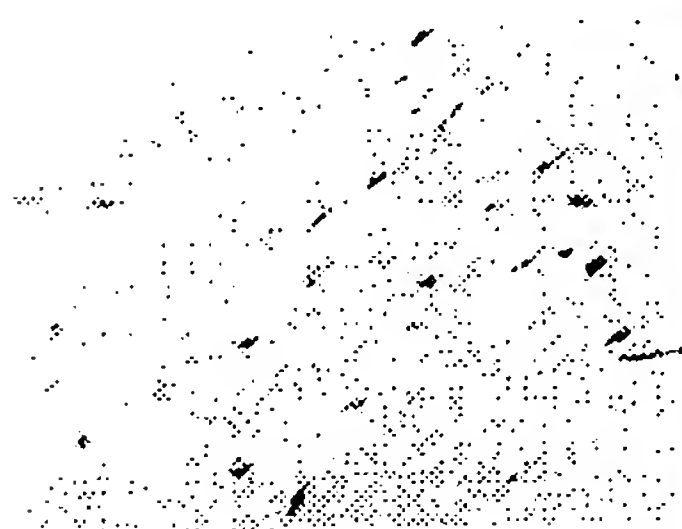


FIG. 10D



FIG. 10E



FIG. 10F

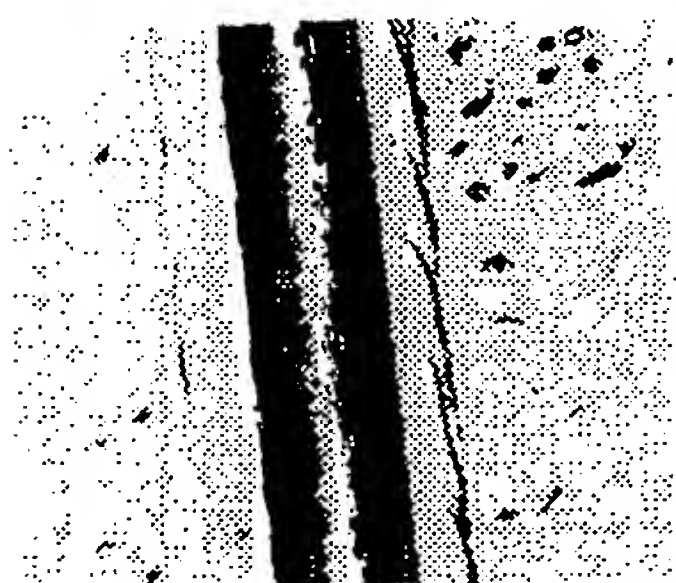


FIG. 10G

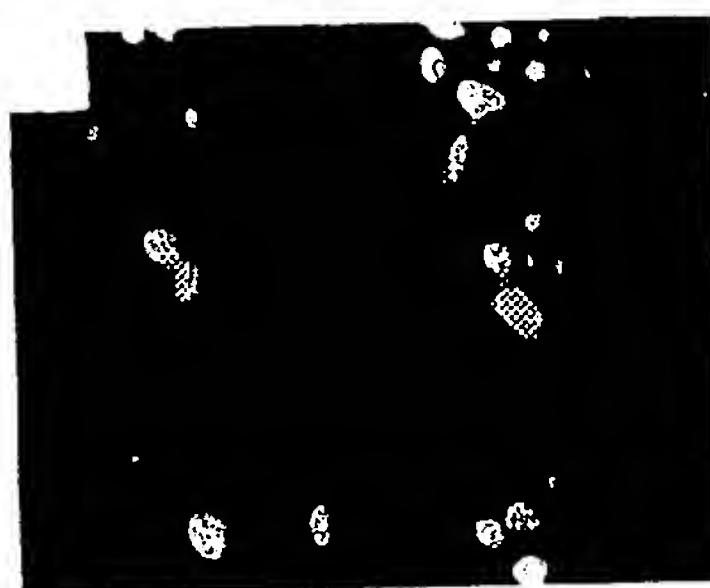


FIG. 10H



FIG. 10I

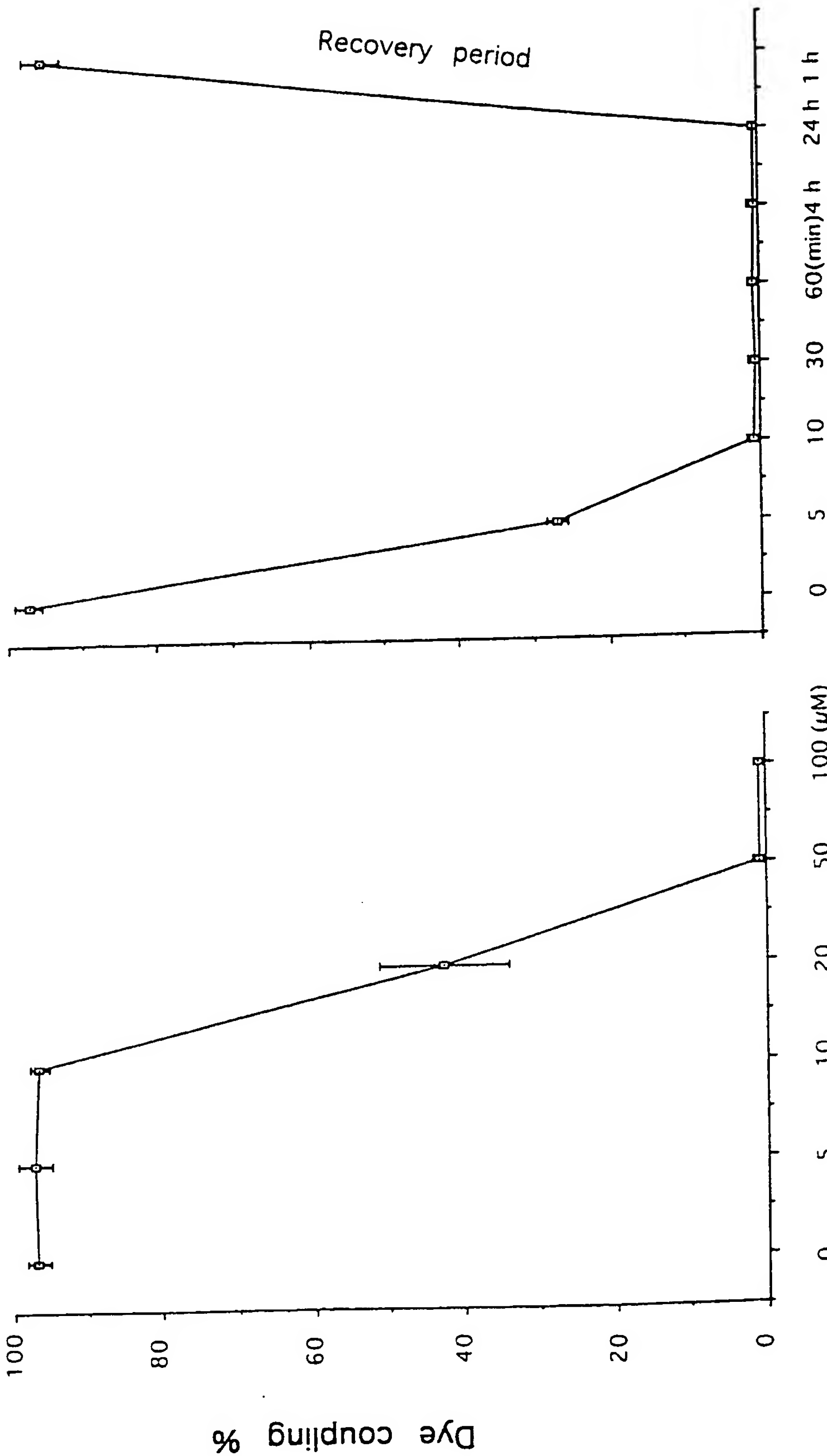


FIG. 11K

FIG. 11J

12 / 22

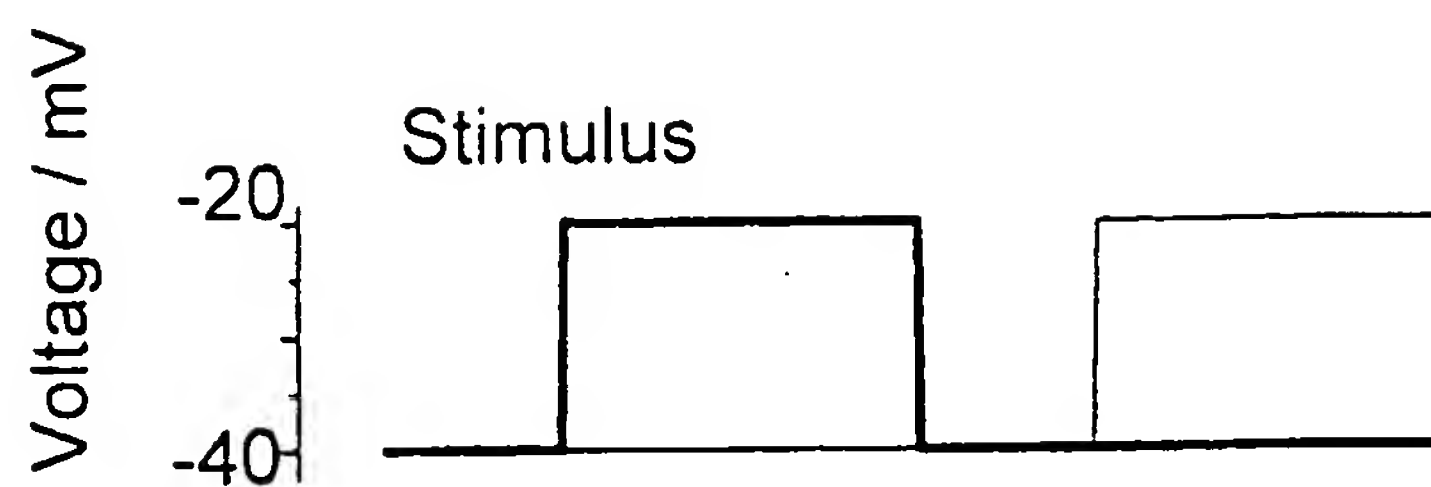


FIG. 12A

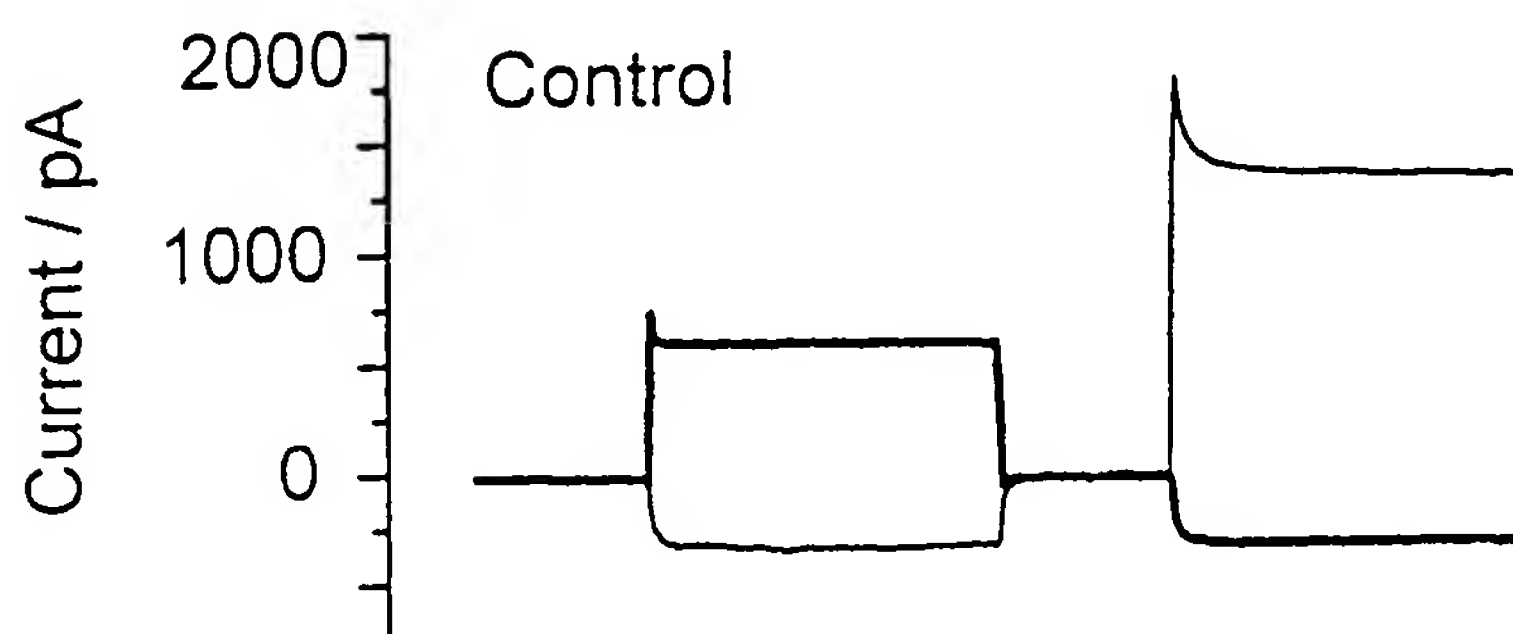


FIG. 12B

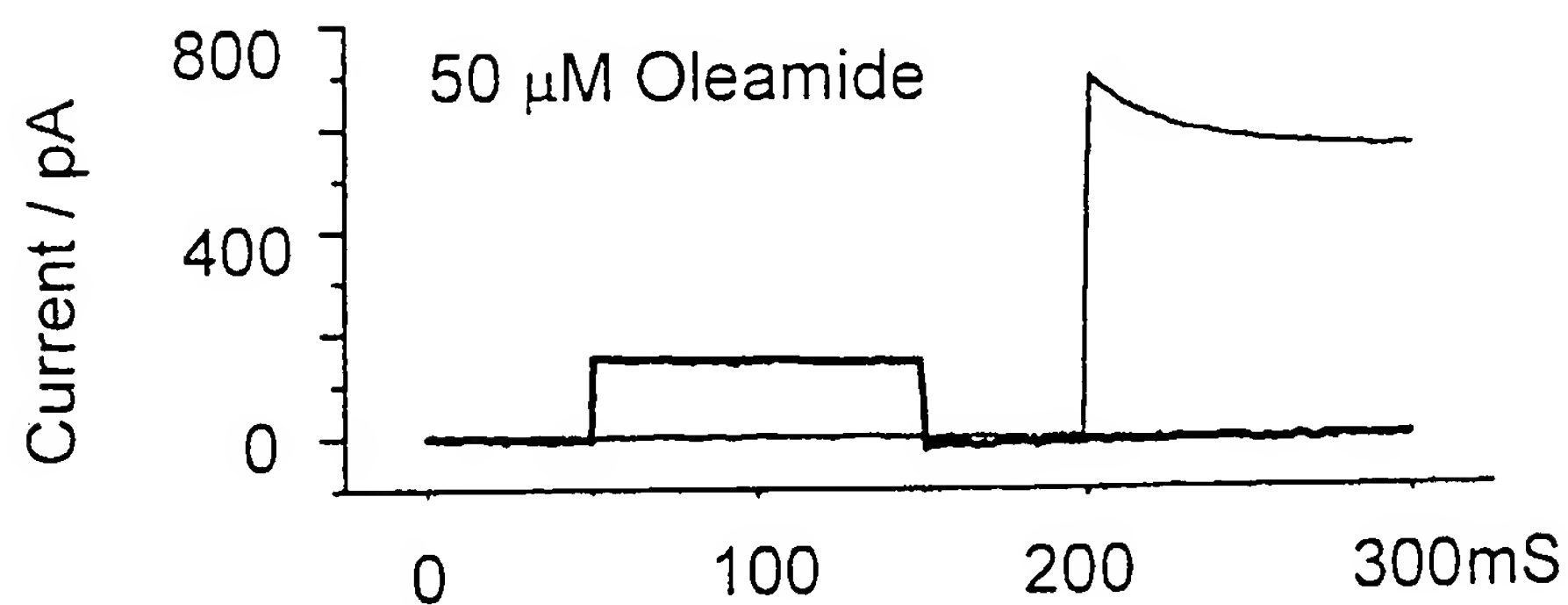


FIG. 12C

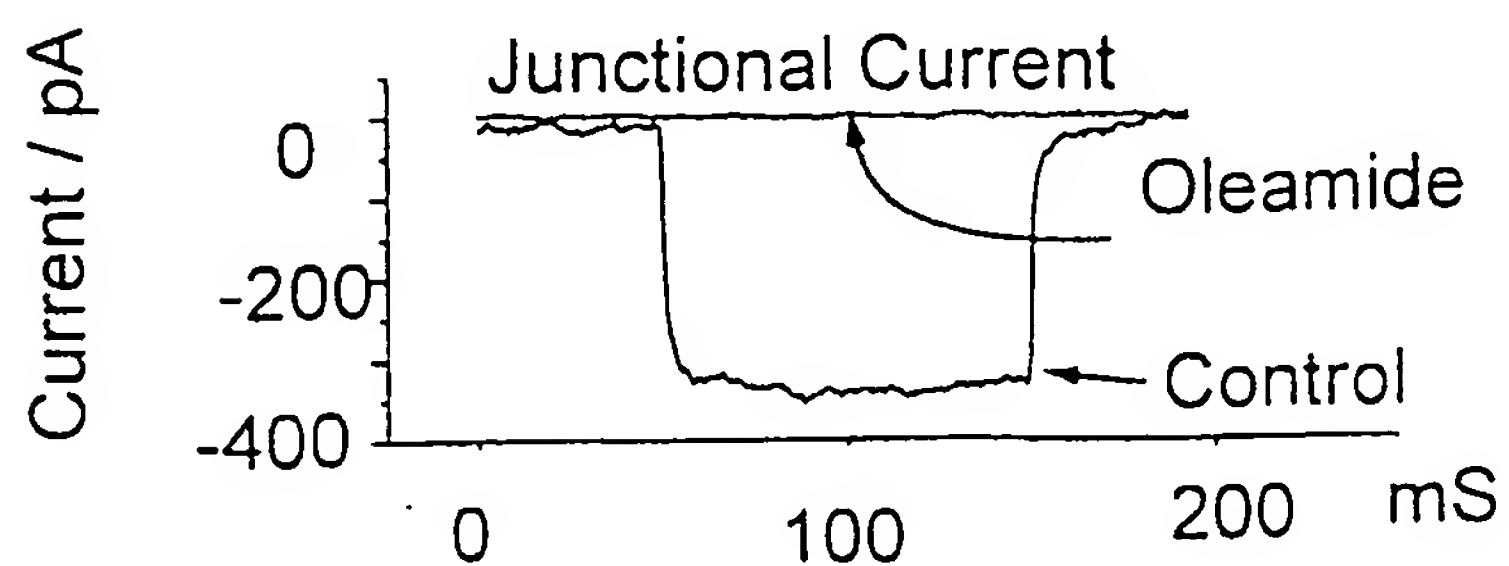


FIG. 12D

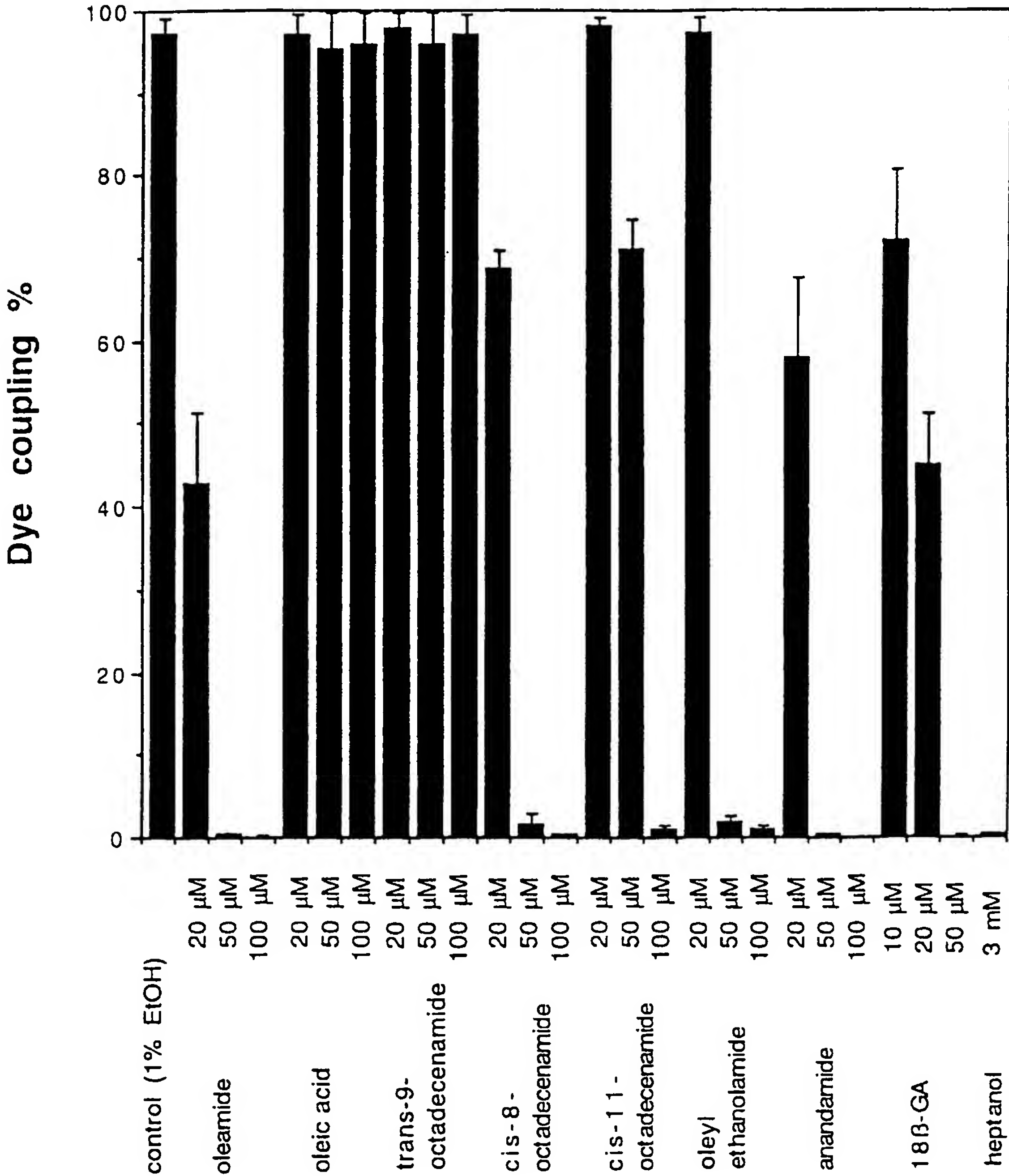


FIG. 13

14 / 22

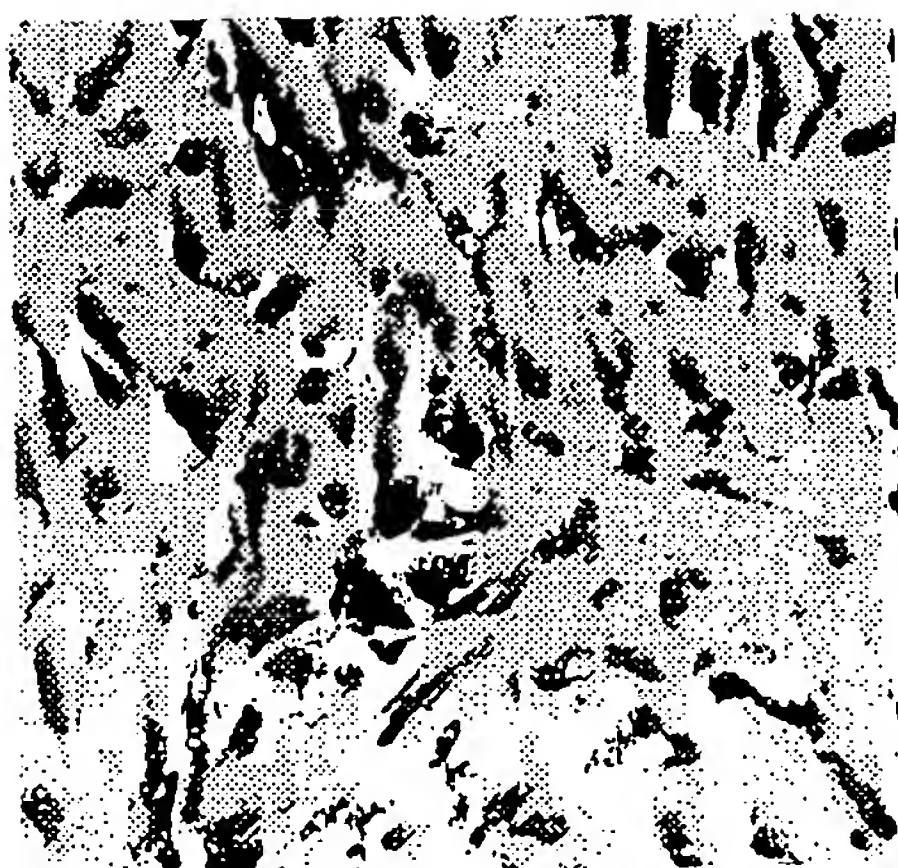


FIG. 14A

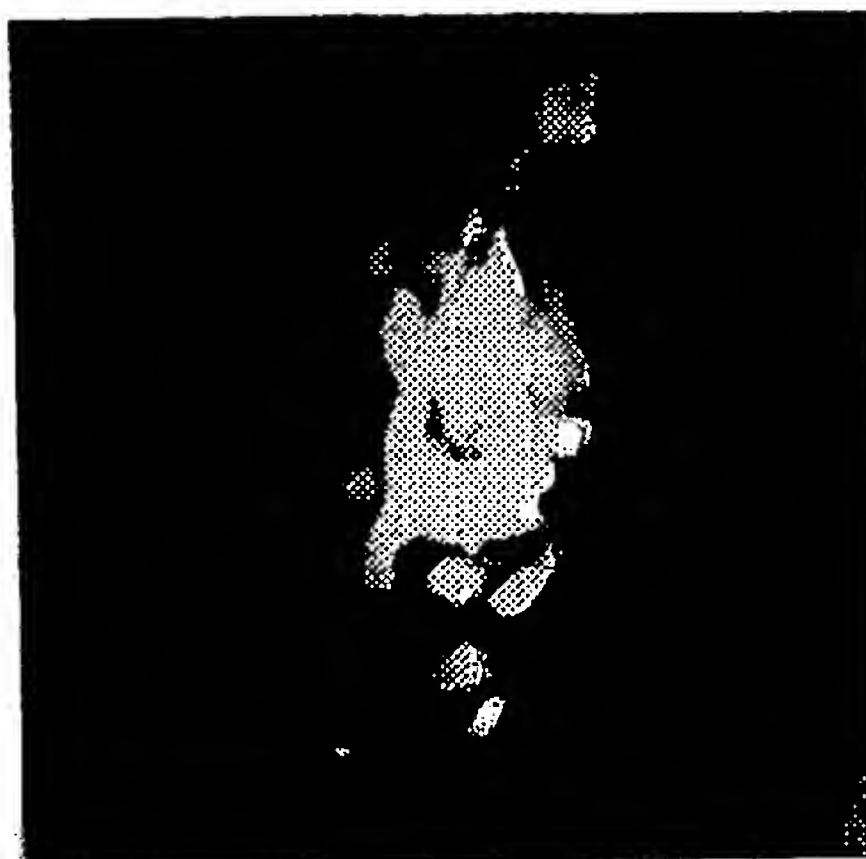


FIG. 14B

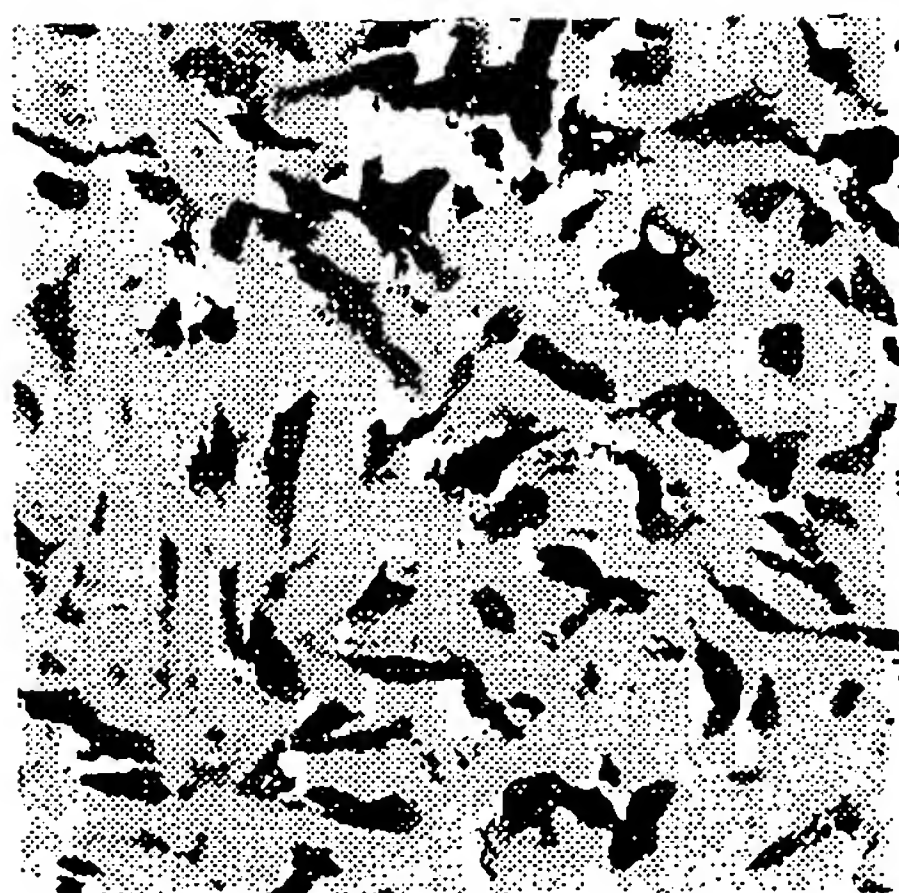


FIG. 14C



FIG. 14D



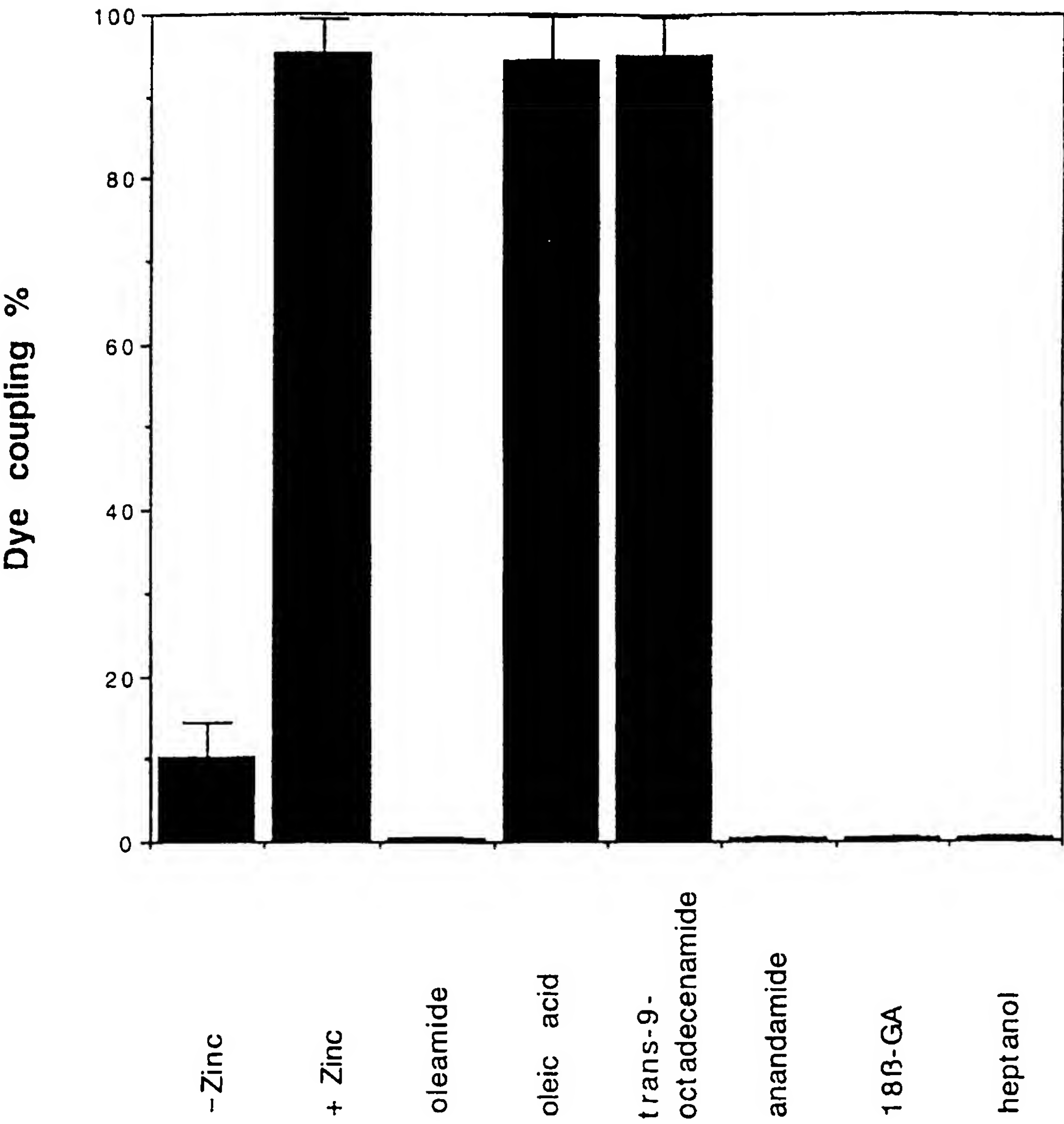


FIG. 15E

16 / 22



FIG. 16A



FIG. 16B

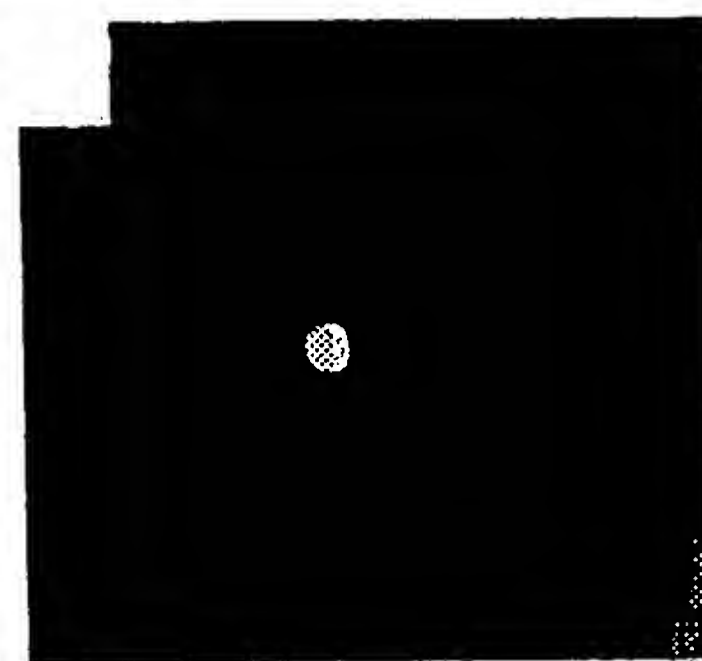


FIG. 16C

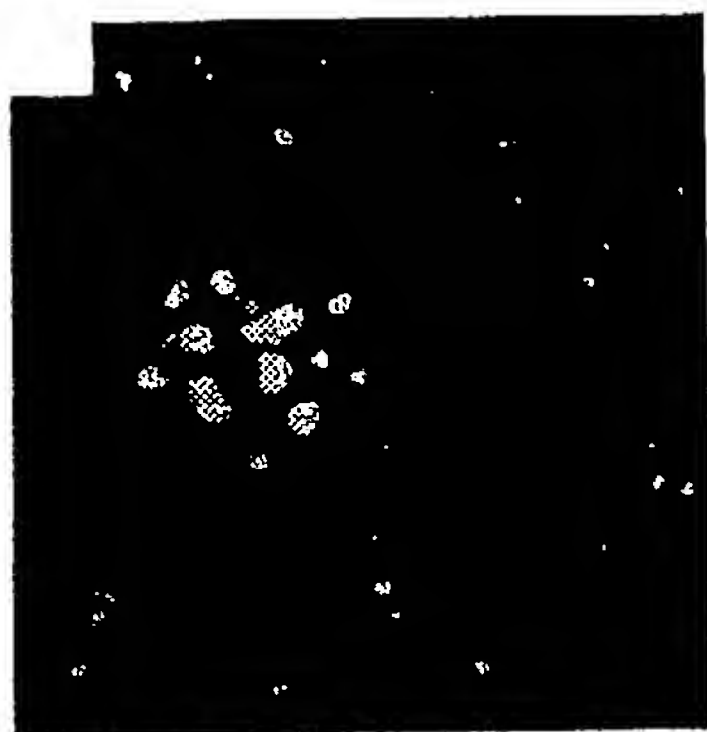


FIG. 16D



FIG. 16E

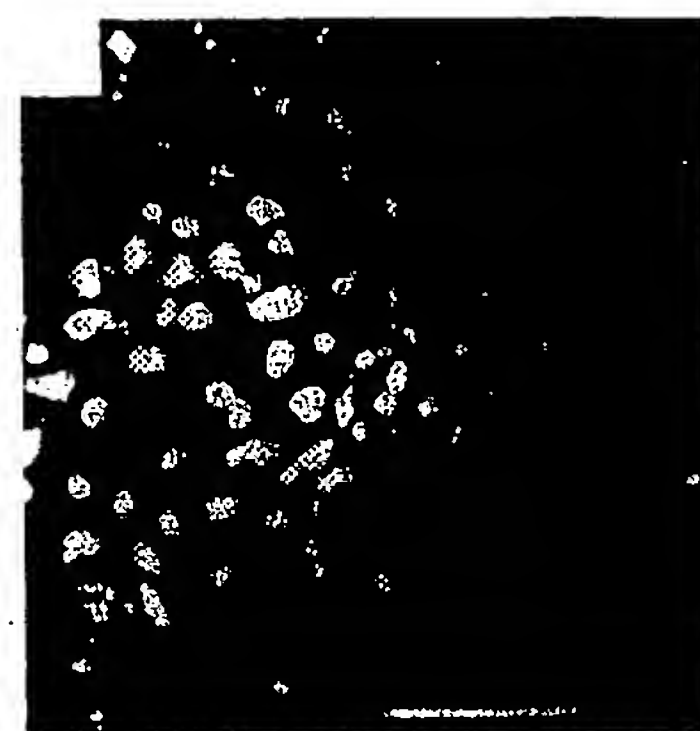


FIG. 16F

17 / 22

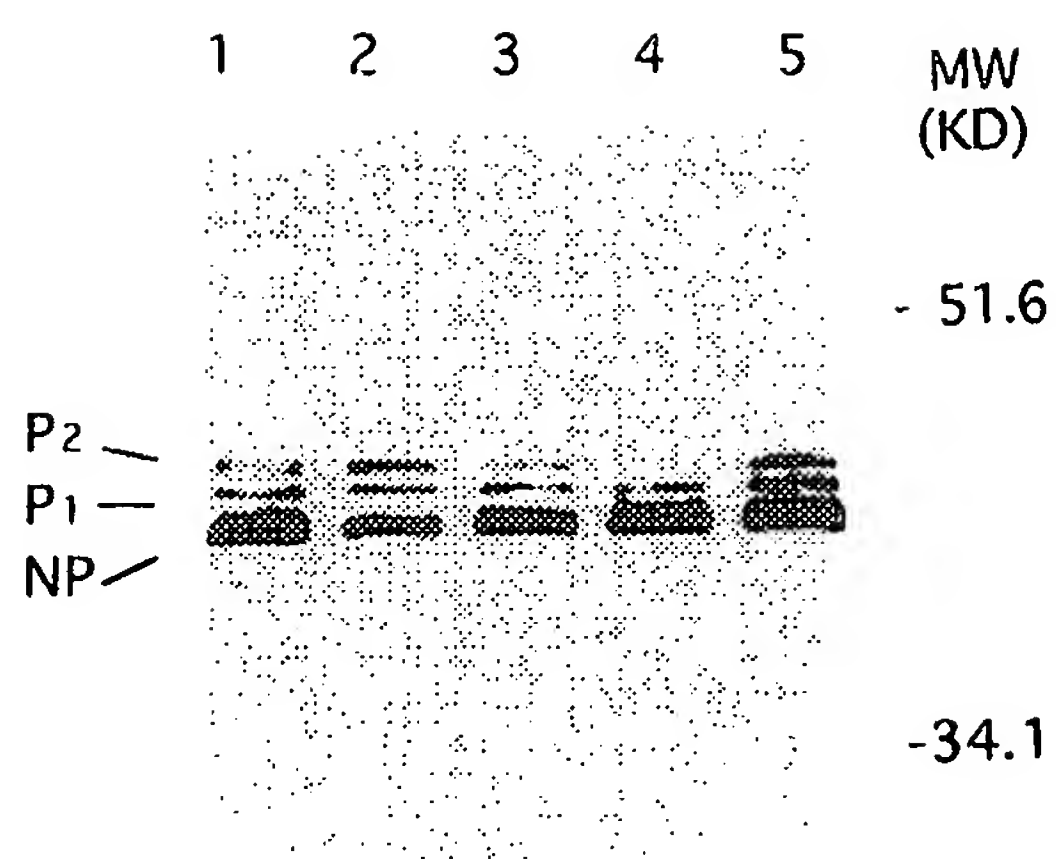


FIG. 17

18 / 22

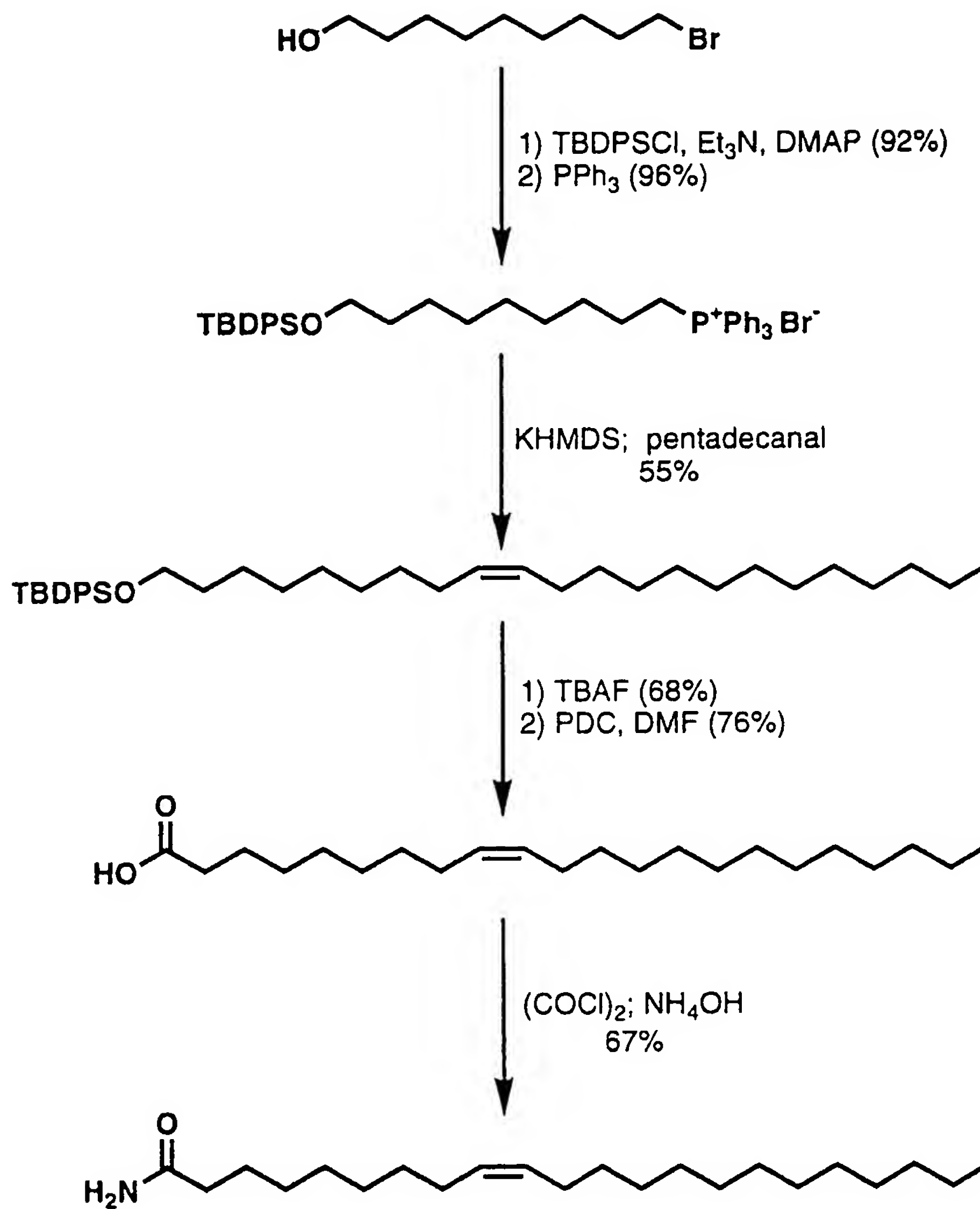
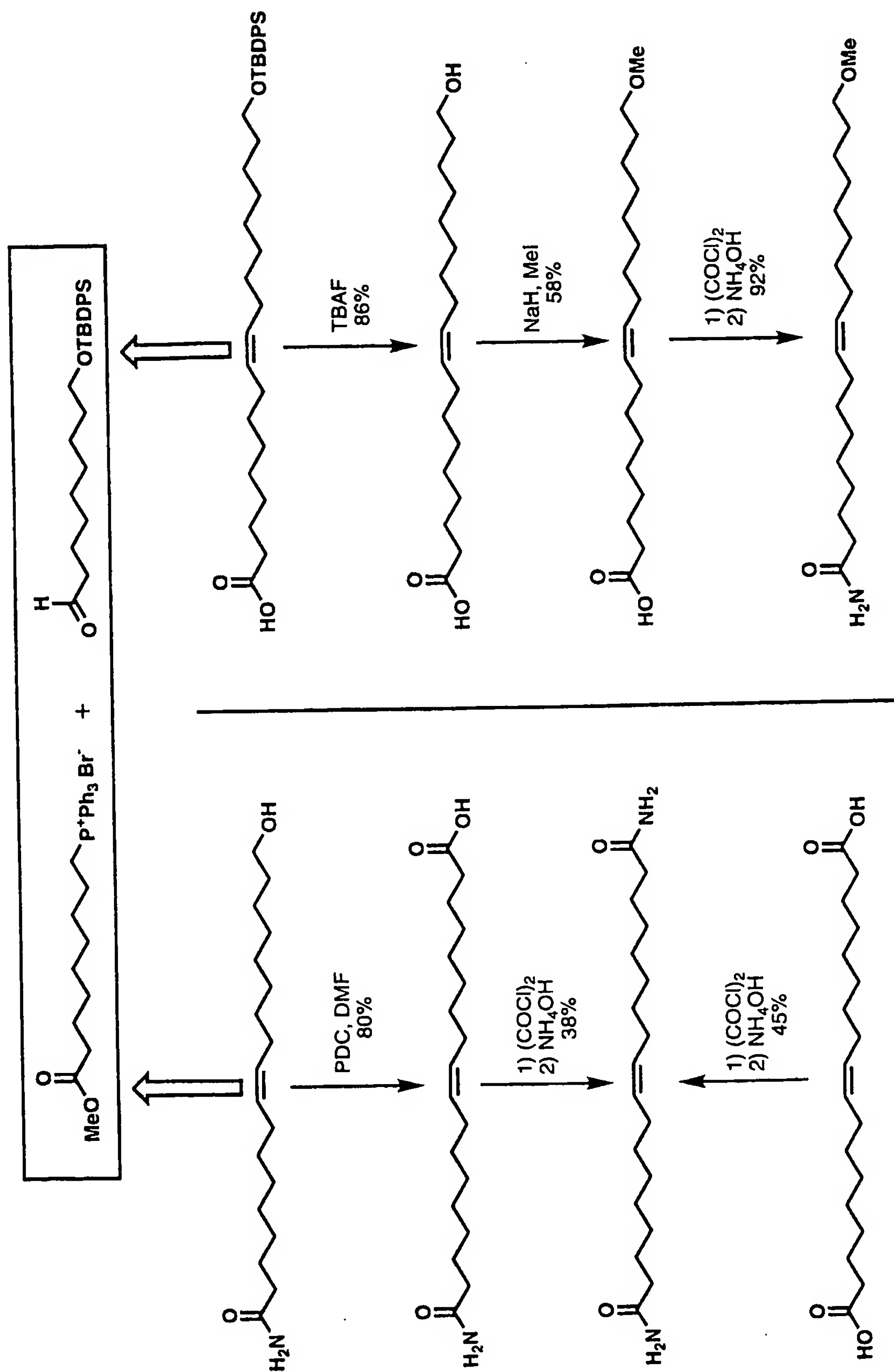


FIG. 18



**FIG. 19**

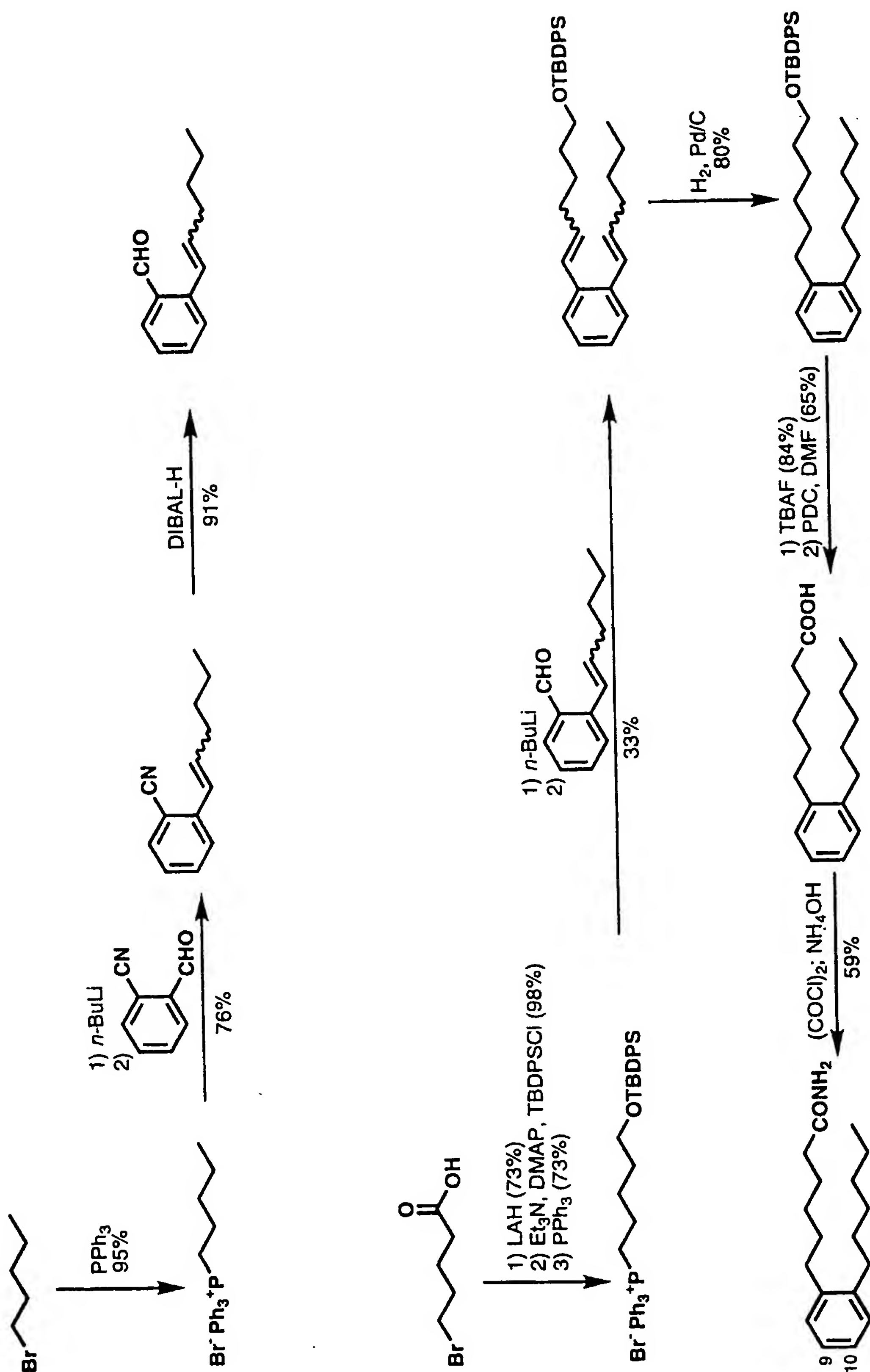


FIG. 20



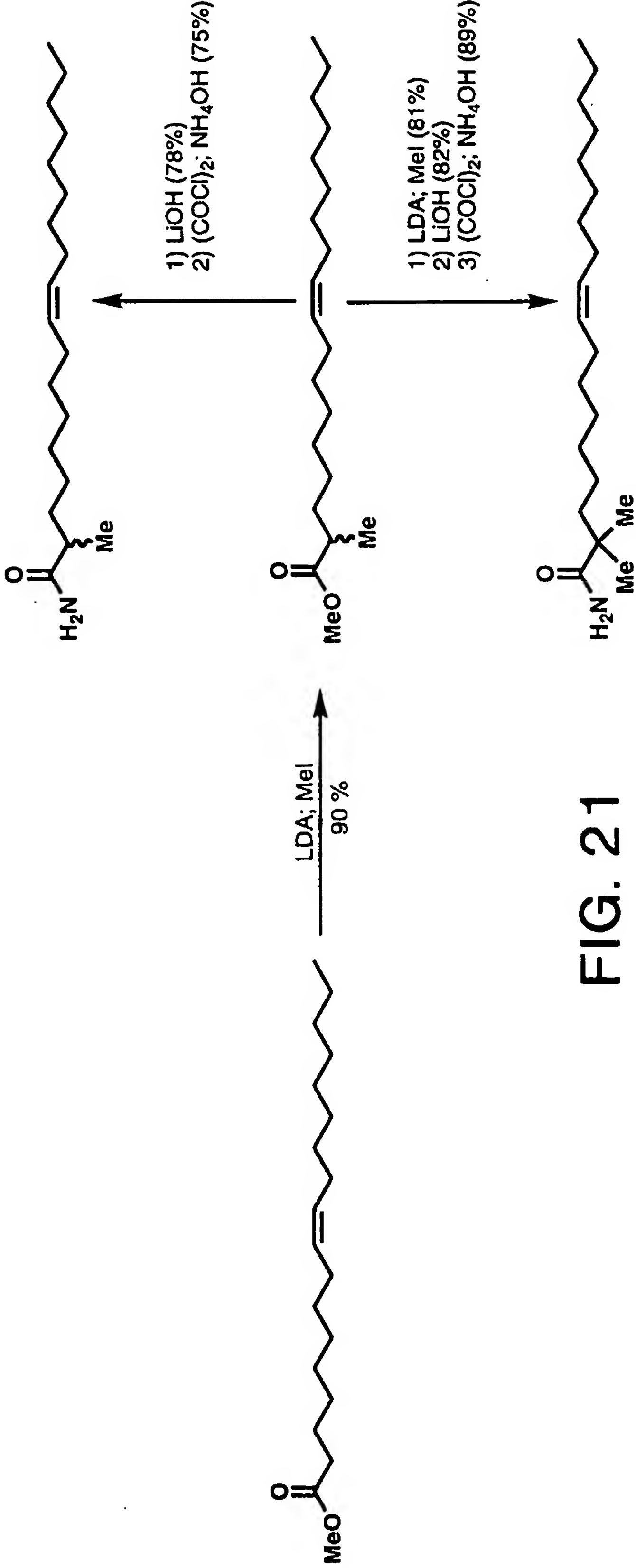


FIG. 21

22 / 22

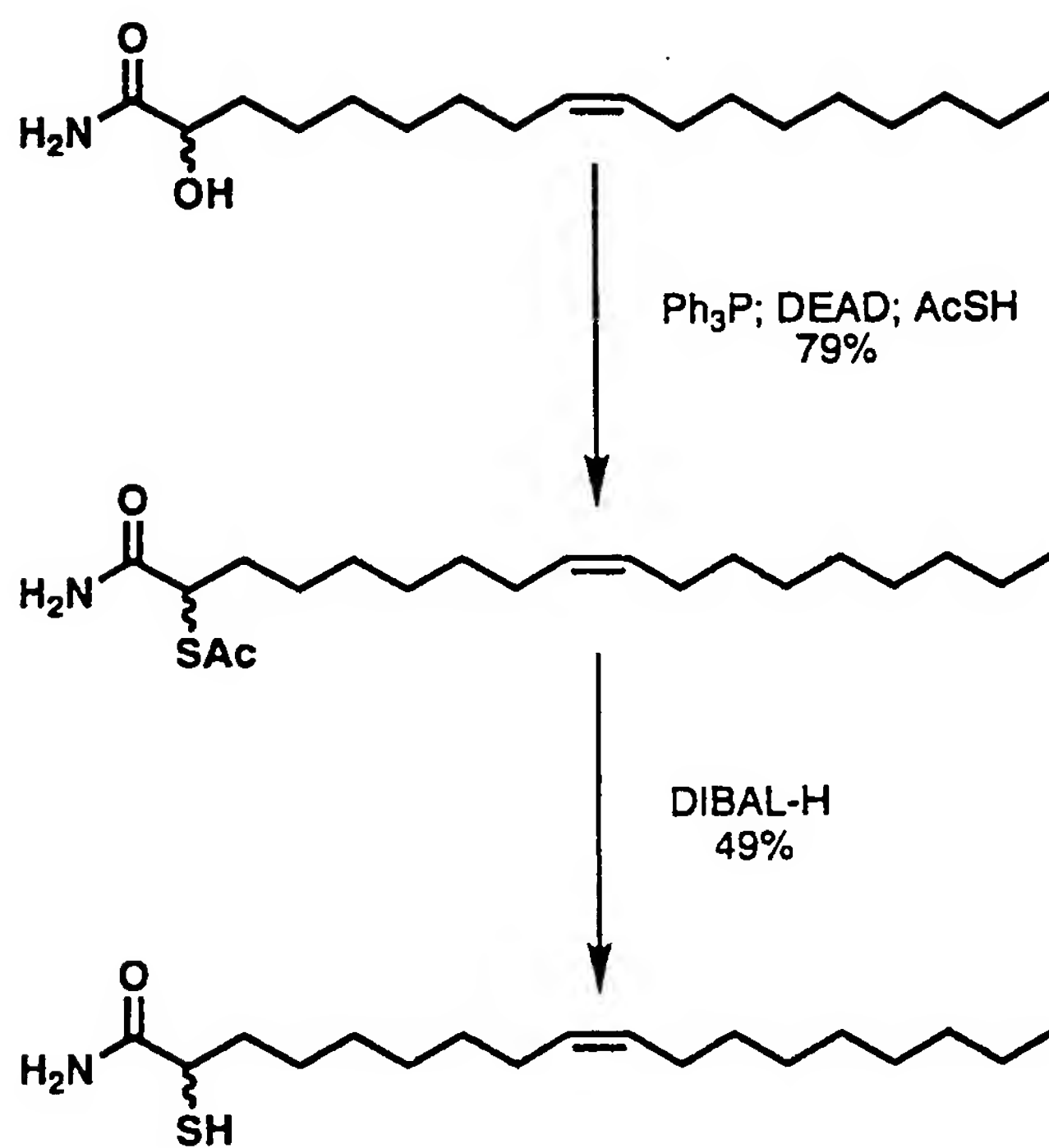


FIG. 22